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THE ANATOMY OF THE EYES AND NEURAL GLANDS IN THE AGGREGATED FORMS OF CYCLOSALPA DOLICHOSOMA-VIRGULA AND SALPA PUNCTATA.[1]

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(WITH PLATES VII., VIII. AND IX.)

The eyes of the solitary salpas of the several species show but little diversity. All are in the form of a horseshoe above the ganglion, the ends of the horseshoe pointing forward. In *Iasis cordiformis-zonaria* the ends of the horseshoe diverge, pointing outward and upward at an angle of about 45°. In *Salpa runcinata-fusiformis* there are masses of somewhat irregular rod-cells in the dorsal part of the ganglion, in front of the eye. With these exceptions the differences in the eyes of the solitary forms of the different species are too minute for verbal description.

In the aggregated forms, on the other hand, the diversity between the eyes is very great. Each species has its own characteristic and distinctive form of eye and the histological differences

[1] This study of the eyes and neural glands of two species of *Salpidæ* was accepted in June, 1903, by the Woman's College of Baltimore, in partial discharge of the requirements for the degree Master of Arts at that time conferred upon Miss Mary E. G. Lentz. Its publication has been delayed until I could receive and study additional material of these and other species of Salpa in the hope of getting conclusive evidence upon disputed points of innervation. I obtained from Naples material of three species of Salpa preserved some in half strength glycerine and some in chloral hydrate, and endeavored to study the innervation of the eye by the use of dissociation media, especially Haller's acetic acid mixture, but the results obtained are not sufficiently conclusive to warrant publication.

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are great. In addition to the large dorsal eye some species have smaller eyes in the ganglion, and the number, position and character of these smaller eyes vary with the species. It is, therefore, important that we have recorded descriptions of the anatomy of the eyes in the chain individuals of all species. Especially is this true since suggestions as to relationship, so readily received from the study of the eyes, appear to be trustworthy.

The neural glands of the chain forms of different species may also be very diverse, making it desirable to have descriptions of their anatomy in all species.

In the present paper the anatomy of the eyes and of the neural glands will be described for the chain forms of two species whose eyes have not before been studied—*Clycosalpa dolichosoma-virgula* and *Salpa punctata*.

The other salpas the anatomy of whose eyes has been studied are :

Cyclosalpa pinnata, solitary and chain forms (by Göppert and Metcalf).

C. chamissonis, chain form (by Metcalf).

Salpa africana-maxima, solitary and chain forms (by Göppert and Metcalf).

S. runcinata-fusiformis, solitary and chain forms (by Göppert and Metcalf).

S. cylindrica, solitary and chain forms (by Metcalf).

Iasis hexagona, solitary and chain forms (by Metcalf).

I. costata-tilesii, solitary and chain forms (by Metcalf).

I. cordiformis-zonaria, solitary and chain forms (by Metcalf).

Pegea scutigera-confederata, solitary and chain forms (by Göppert and Metcalf).

P. scutigera-confederata var. *bicaudata*, chain form (by Metcalf).

Thalia democratica-mucronata, solitary and chain forms (by Göppert and Metcalf).

The anatomy of the neural glands has been described by Metcalf for the chain and solitary forms of the species mentioned in the foregoing list, except that the gland has not been described for the solitary *Pegea scutigera-confederata* var. *bicaudata*. It is probable that *bicaudata* is a variety of the chain form only and that its solitary form resembles that of *scutigera-confederata*.

The development of the eyes and neural glands has been described by Metcalf for *Cyclosalpa pinnata*, chain and solitary forms.

CYCLOSALPA DOLICHOSOMA-VIRGULA, chain form.

The condition of the eye in this species is of interest because it forms the third link in a series of three conditions bridging over the gap between the *Cyclosalpæ* and the true *Salpæ*. The structure of its neural glands is not unique.

The brain of *Cyclosalpa dolichosoma-virgula* is a ganglion of almost spherical shape (Fig. 1). Two outgrowths from the ventral surface, one on either side of the mid-line, extend to the neural glands whose position is ventro-lateral to the ganglion. These glands, with their long lateral ducts, are partially shown in Fig. 1 (*h*, gland; *d*, duct). On the postero-dorsal surface of the ganglion is a slight protuberance (Fig. 5, *pr*). All the nerves, with the exception of a single pair, spring from the equatorial zone of the ganglion (Fig. 1). One pair arises from the ventral outgrowths. The nerves are probably constant in number for the species — forty-eight.

The large ovoid eye (Fig. 1) projects anteriorly from the posterior end of the dorsal surface of the ganglion, and the long axis of the eye makes with this surface an angle of about 30° (Fig. 5). The eye points about 40° from the mid-line of the body, to the right or left, according as the animal lies to the right or left in the chain. One third of the eye, approximately, extends beyond the anteriormost edge of the ganglion.

There is a deep invagination of the ectoderm into the narrow space between the eye and the ganglion, and a less abrupt invagination just posterior to the ganglion (Fig. 5). In the ectodermal chamber formed between these two invaginations lies the eye, which presses closely against the ectoderm only at its tip. Blood sinuses (Fig. 1, *bl*) which surround the ganglion are continuous with this optic chamber (Fig. 5, *oc*).

The ganglion has the usual fibrous core, and a peripheral cellular layer (Fig. 5), which extends inward about one seventh of the diameter. The postero-dorsal protuberance (Fig. 5, *pr*), is made up of ordinary ganglionic cells. Large nerve cells (Fig. 5, *nc*), lie in the equatorial zone from which the nerves arise.

These large cells lie among the smaller ganglionic cells of the peripheral layer. A large bundle of nerve fibers, arising in the fibrous core of the ganglion, passes through the peripheral cellular layer, just anterior to the protuberance on the posterior surface, and enters the eye at its posterior end, forming the optic nerve (Fig. 5, *on*). Fibers ramify also among the cells of the postero-dorsal protuberance (Fig. 5, *pr*).

Four kinds of cells are found in the large eye, and one of these, so far as known, is peculiar to this species. The pigment cells and rod-cells resemble the characteristic cells found in the well-developed eyes throughout the genus. The pigment cells cover the dorsal surface of the eye from near the tip to slightly beyond the middle (Fig. 1 and Fig. 5, *dp*). They then extend around the lateral surfaces in posteriorly-directed broad bands, uniting on the ventral surface to form a pigment layer which extends from beyond the middle of the eye to the base (Fig. 5, *vp*). The position of the dorsal and ventral pigment is also shown in cross sections (Fig. 7, *dp*; Fig. 9, *vp*). A cross section through the middle of the eye (Fig. 8), shows the complete enwrapping of this portion of the eye by pigment. The pigment cells lie outside the optic membrane (Fig. 5, *oz*), which is a continuation of the ganglionic membrane. They are therefore probably mesodermal.

The rod-cells are elongated cells with large nuclei (Fig. 5, *dr* and *vr*). They have thick-walled basal ends and thin-walled tips. In the anterior and posterior regions of the eye the thick-walled ends of the rod-cells are toward the pigment (Figs. 5, 7 and 9). In the center of the eye, where the pigment forms a continuous enveloping layer, the thick-walled inner ends of the rod-cells have a somewhat confused arrangement, while their thin-walled tips point toward the pigment cells (Figs. 5 and 8).

The third kind of cells present in the eye are similar to the ordinary cells of the ganglion, having nuclei of about the same size. These cells, which have been called intermediate cells, lie between the thick-walled ends of the anterior and posterior rod-cells and the pigment cells (Fig. 5, *i* and *i'*; Fig. 7, *i*; Fig. 9, *i'*). Intermediate cells are not present in all species. In the chain forms of *Cyclosalpa pinnata* and *Pegea scutigera-confederata* the rod-cells abut directly on the pigment layers.

The large optic nerve, when it enters the eye, passes directly above the thin-walled ends of the rod-cells in the posterior portion of the eye (Fig. 5; Fig. 9, *on*). The nerve can easily be traced as far as the middle of the eye, but the course of the fibers beyond that point could not be satisfactorily determined. In sections through the tip of the eye, the nerve fibers seem to appear ventral to the rod-cells (Fig. 5), and it seems probable that they enter these cells at their thin-walled ends; but the shrinking of the protoplasm from the tips of the rod-cells, apparently due to the action of the preserving fluids, makes the rod-cell walls more clearly visible, so that it is very difficult to distinguish between these cell boundaries and probable innervating fibers. Sufficient evidence was not obtained to warrant a categoric denial of Göppert's assertion that the rod-cells in the anterior portion of the eye, in the chain forms, receive their fibers at their thick-walled ends, though the indications are all against this belief. To conclusively decide this disputed point of innervation, attempts were made at macerating preserved material, but without success. Any one able to obtain fresh specimens could probably determine readily by maceration the manner of innervation. Best suited for this purpose are the following species, in which the rod-cells are well-developed: *Cyclosalpa pinnata*, *Cyclosalpa chamissonis*, *Cyclosalpa dolichosma-virgula*, *Salpa runcinata-fusiformis*, *Salpa africana-maxima*, *Salpa cylindrica*, *Thalia democratica-mucronata*, *Salpa punctata*.

The fourth kind of cells, found in the eye of the chain form of *Cyclosalpa dolichosoma-virgula*, are those which have been mentioned as perhaps peculiar to this species. They lie in a single group, which is partially imbedded among the pigment cells of the mid-dorsal region (Fig. 5, *q*; Fig. 8, *q*; enlarged, Fig. 10, *q*). These cells are spindle-shaped, and have nuclei about the size of the intermediate-cell nuclei. They are inclined at an angle of about 45° to the long axis of the eye (Fig. 10), and they are separated from the posterior rod-cells by the optic nerve fibers. The anteriormost cells of the group are wholly surrounded by pigment (Fig. 8, *q*). There seems to be some indication that these spindle-shaped cells receive at their posterior ends innervating fibers from the optic nerve. A probable homol-

ogy of this group of cells with certain portions of the eye of *Cyclosalpa pinnata* and *Cyclosalpa chamissonis* will later be discussed.

Small groups of apparent rod-cells are found in the peripheral cellular layer of the ganglion, (Fig. 5, *ey*, *ey'*, *ey''*). They may perhaps be called small eyes, because they are made up of cells with thick-walled basal ends, similar to the rod-cells of the large eye. An enlarged drawing of one of these groups is shown in Fig. 11. The cells of these small eyes are not elongated as are the rod-cells of the large eye, and their nuclei are the same size as those of the ordinary ganglionic cells. More or less similar smaller eyes occur in the ganglia of the chain forms of *Cyclosalpa pinnata*, *C. chamissonis*, *Salpa cylindrica*, *S. runcinata-fusiformis*, *Iasis hexagona*, *I. costa-tilesii*, *Pegea scutigera-confederata* and *Thalia democratica-micronata*. Compare Fig. 18, Plate IX., which shows their position and appearance in *Cyclosalpa pinnata*.

There is no pigment present in these small eyes of any species described except *Salpa costata-tilesii*. Göppert assumes that these eyes are functional optic organs, but the absence of pigment makes the correctness of this assumption doubtful.

The small groups of rod cells which occur in the ganglion of *Cyclosalpa dolichosoma-virgula* vary in number and position, but all have about the same structure. Their cells are about the same size as the ordinary peripheral cells of the ganglion and seem to be developed from these merely by the formation of the peculiar outer glassy layer indicated in the figures (Plate VIII., Figs. 5 and 11) by the heavy black lines. The glassy outer portion of one of these cells resembles in histological character the glassy outer layer, which we have called the thickened cell-wall, seen at one end of any rod-cell of the larger eye, but the cells are of quite different shape, being spheroidal, or irregularly polyhedral, instead of cylindrical. There seems to be a general tendency in the chain forms of the different species of *Salipidæ* to form from the smaller cells of the ganglion such groups of imperfect rod-cells.

Each of the two latero-ventral outgrowths which push out from the ganglion toward the glands consists of cells of two sizes.

The ordinary ganglionic cells form that portion next to the ganglion (Fig. 6, *b*). A distinct though thin membrane then intervenes (Fig. 6, *gz*), separating the small-celled portion from a large-celled area (Fig. 6, *b'*), which extends to the wall of the gland (Fig. 6, *gw*), and is separated from the gland by a thick membrane (Fig. 6, *hz*). The thin membrane, which intervenes between the small and large cells of the outgrowths is continuous with the delicate membrane that surrounds the ganglion. The neural glands and their lateral ducts have walls made up of a single layer of cells, except that the wall of that side of each gland which lies next to the ganglionic outgrowth, is composed of several layers of cells (Fig. 6, *gw*). These conditions are very similar to those described for *Cyclosalpa pinnata.*

Salpa Punctata.

The ganglion of the chain form of *Salpa punctata* (Fig. 12) differs in shape from the nearly spherical ganglion of *Cyclosalpa dolichosoma-virgula.* Its dorso-ventral axis is about one and one half times the length of its anterior-posterior and transverse axes. Twenty-seven nerves were counted (Fig. 2). As in *Cyclosalpa dolichosoma-virgula*, they all arise from the equatorial zone of the ganglion, with the exception of a single pair from the ventral outgrowths. The average thickness of the peripheral cellular layer (Fig. 12), is about one twelfth of the ganglion's mean diameter.

In a dorsal position, just beneath the ganglionic membrane, is a double layer of rod-cells, with the thickened ends of the cells of one layer abutting upon the thickened ends of those of the other layer (Fig. 12, *ex*). These cells are not as elongated as the rod-cells of the large eye, but their bulk is about the same and their nuclei are about the same size. There are no scattered groups of small rod-cells in the peripheral cellular layer of the ganglion of *Salpa punctata*, such as have been described for *Cyclosalpa dolichosoma-virgula.* This group of rod-cells in the dorsal part of the ganglion of *Salpa punctata* probably belongs to the same category as the small eyes found in the ganglia of many species. The presence of many sorts of these small groups of rod-cells in different positions in different species renders homologies between them doubtful.

The large eye projects from the antero-dorsal face of the ganglion, and therefore lies almost wholly anterior to the ganglion. Its projection to the right or left of the mid-line of the body is slightly less than that of the eye of *Cyclosalpa dolichosoma-virgula*. The eye is relatively short, and tapers but slightly toward the tip. The dorsal pigment layer extends more than half way down over the tip (Figs. 12 and 13, *dp*), thus enveloping the dorsal half of the anterior portion of the eye in a hood of pigment. The lateral posteriorly-directed pigment bands (Fig. 15, *lp*), meet at the postero-ventral angle of the eye to form the ventral pigment surface, which is of less extent than the dorsal (Fig. 12, *vp*). Slightly posterior to the middle of the eye the dorsal pigment dips down forming a pigment curtain (Fig. 12, *pc*), which extends nearly half way through the eye, and is laterally continuous with the lateral pigment layers (Fig. 14). This pigment curtain is slightly dome-shaped with the apex of the dome toward the anterior. Fig. 14 shows a section through the pigment curtain just posterior to its apex.

The rod-cells have the same general arrangement as in *Cyclosalpa dolichosoma-virgula*, but in *Salpa punctata* those pointing ventrally (Fig. 12, *vr*) exceed in number those pointing dorsally (Fig. 12, *dr*). This corresponds to the fact already mentioned that the greater amount of pigment is on the dorsal surface. In the middle of the eye, where it is entirely enwrapped by pigment, the rod-cells point both dorsally and ventrally, with their thick-walled ends central. None point laterally, as in *Cyclosalpa dolichosoma-virgula* (compare Fig. 8, *lr*). The optic nerve enters the eye above the thin-walled tips of the posterior rod-cells (Fig. 12, *on*), and the innervation is believed to be the same as that of *Cyclosalpa dolichosoma-virgula*.

A large group of intermediate cells lies between the dorsal pigment and the ventrally-directed rod-cells (Fig. 12, *i*). A smaller group lies between the ventral pigment and the few dorsally-directed rod-cells (Fig. 12, *i'*). In *Cyclosalpa dolichosoma-virgula* the intermediate cells are confined to those two regions, but in the *Salpa punctata* the intermediate cells extend also beneath the lateral pigment layers. The position of the lateral intermediate cells in *Salpa punctata* is the same as that of the

laterally-directed rod-cells of *Cyclosalpa dolichosoma-virgula* (compare Fig. 8, *lr*), and these cells in the two species are probably homologous, the rod-cells in *Cyclosalpa dolichosoma-virgula* having developed from intermediate cells. A few intermediate cells lie posterior to the dorsal pigment curtain (Fig. 12, *q'*). The group of spindle-shaped cells in the eye of *Cyclosalpa dolichosoma-virgula* occupies a similar position (compare Fig. 5, *q*).

The ectoderm (Fig. 12, *os*), closely covers the entire eye and dorsal surface of the ganglion, except at the postero-dorsal angle of the ganglion. A membrane (Fig. 12, *z*), continuous with the ganglionic membrane, meets the ectoderm posterior to the ganglion, shutting off a small chamber (Fig. 12, *oc*), which is homologous with the posterior portion of the optic chamber of other species, such as *Cyclosalpa dolichosoma-virgula*. The limiting membrane of the optic chamber (Fig. 12, *z*), described for *Salpa punctata* is not found in *Cyclosalpa dolichosoma-virgula*. It is present in many other species, and its absence in *Cyclosalpa dolichosoma-virgula* is exceptional.

The ectoderm, after covering the eye and ganglion, turns abruptly dorsalward, so that the eye and ganglion lie just under an ectodermal invagination. This invagination thus forms a supra-neural ectodermal chamber which is filled by an unusually dense portion of the tunic (Fig. 12, *sc*). It has a somewhat triangular dorsal opening to the exterior. The supra-neural ectodermal chamber in *Salpa punctata* probably serves for the eye and ganglion a similar protective purpose to that of the large optic chamber of *Cyclosalpa dolichosoma-virgula*. A similar ectodermal invagination is found above the ganglion of the immature *Salpa runcinata-fusiformis*, chain form.

The latero-ventral outgrowths from the ganglion to the neural glands are similar in structure to those of *Cyclosalpa dolichosoma-virgula*, but smaller. The wall and duct of each gland are composed of a single layer of cells. That portion of the wall next to the ganglion is thickened by the elongation of the cells but these cells even here form a single layer. This is unlike the condition found in *Cyclosalpa dolichosoma-virgula*, in which the thickening of the gland wall next to the ganglion is caused by the presence of several layers of cells. The gland ducts of

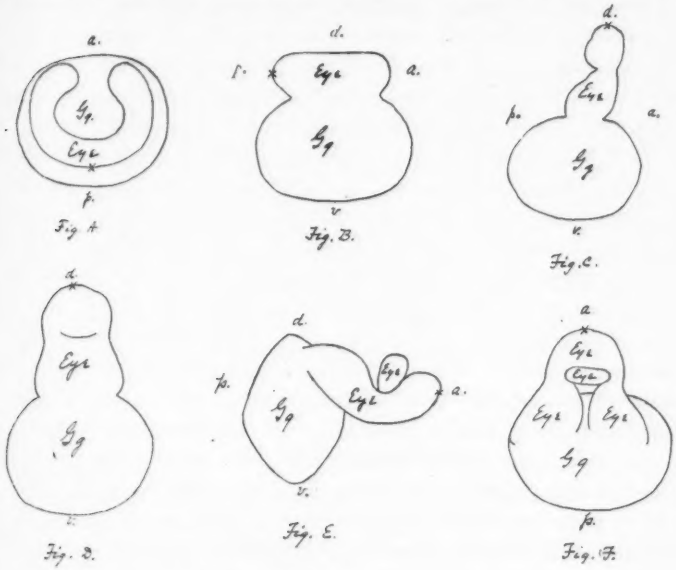
Salpa punctata are very broad and flattened, and show in cross-section a slit-like lumen. They are convoluted at their distal apertures (Fig. 16).

The eyes in the three cyclosalpas, *pinnata*, *chamissonis*, and *dolichosoma-virgula*, show an interesting series of modifications. Figs. 3 and 4, Plate VII., show the character of the large eye in *C. pinnata*. It is roughly a horseshoe with the ends directed backward, and having an additional mass of optic cells placed in the curve of the horseshoe. This eye has been shown (by Metcalf) to be derived from a horseshoe-shaped eye, like that of the solitary salpa, whose free ends point forward. The position of the eye has been reversed by an actual tipping forward of the whole eye, the ends of the horseshoe remaining attached to the ganglion, while the curved part of the horseshoe swings forward through an arc of 180° (*cf.* Text-figs. 1 to 6). The horseshoe shape of the adult eye of the chain *C. pinnata* is therefore probably in a sense primitive, a reminder of the condition seen in the embryo, which corresponds to that in all the solitary salpas. For a time, however, in the development of the chain *C. pinnata* the young eye is undivided, there being no split between the lateral halves. This split, which appears later, is probably a reopening of the earlier space between the limbs of the horseshoe seen at a time when in the young embryo the eye had a form similar to that of the eye of the adult solitary salpa.

Metcalf has shown that the eye of the chain *Cyclosalpa chamissonis* is in a condition a little less developed than that of *C. pinnata*. It is in the form of a flat plate, with no split between its two halves (Pl. IX., Fig. 19), though there are two enlargements of its posterior portion, corresponding to the two limbs of the eye of *C. pinnata* and separated by a furrow which is in the position occupied by the split in the eye of *C. pinnata*. *C. chamissonis* has also an accessory mass of optic cells dorsal to the anterior part of the eye in the same position in which the similar body is seen in *C. pinnata* (compare Fig. 19, r''' with Fig. 18, p''' and e'''). The eye of *C. chamissonis* is either less developed or has reverted to a slightly simpler condition.

The eye of *Cyclosalpa dolichosoma-virgula* (Pl. VII., Fig. 1, and Pl. VIII., Fig. 5) is still more different from that of *C. pinnata*. It

is irregularly conical rather than flat and shows no sign of division into right and left limbs, approaching in form and structure the eye of the true *Salpæ*, for example *S. runcinata-fusiformis*.



Outline drawings of ganglia and eyes of salpa.

a. = anterior; *d.* = dorsal; *Gg.* = ganglion; *p.* = posterior; *v.* = ventral; *x.* indicates in each case the same region of the eye.

FIG. A. Dorsal view of ganglion and eye of a solitary salpa. The horseshoe-shaped eye is above the ganglion, with the ends of the horseshoe pointing forward.

FIG. B. The same seen from the right side.

FIG. C. Ganglion and eye of an immature chain *Cyclosalpa pinnata*. In an earlier condition this eye was horseshoe-shaped and lay horizontally on the dorsal surface of the ganglion, resemblimg Figs. A and B. It has now rotated forward and upward through an arc of about 90°. That surface of the eye which was dorsal when the eye was horizontal is now anterior.

FIG. D. The same eye and ganglion as those shown in Fig. C., but here seen from behind. The eye is in the form of a flat plate, the hollow of the horseshoe having become filled with cells.

FIG. E. The eye and ganglion of an adult, chain *Cyclosalpa pinnata*, seen from the right side. The eye has shifted 90° still further forward and is again horizontal, but with its former posterior portion now anterior. (Cf. Plate VII., Fig. 3.)

FIG. F. Dorsal view of the same eye and ganglion as those shown in Fig. E. The eye is seen to be split posteriorly into two limbs, being again horseshoe-shaped. (Cf. Plate VII., Fig. 4.)

The protuberance from the ganglion, which lies at the base of the large eye, is very similar to that seen in *C. pinnata* (*cf.* Figs. 5 and 18). In the eye of *C. dolichosoma-virgula* there is a group of cells which strongly suggests comparison with the accessory mass of optic cells in the curve of the horseshoe in the eye of the chain *C. pinnata* and with the similar, though less distinct mass of cells in *C. chamissonis*. This mass of peculiar spindle-shaped cells does not show in surface view, but is shown as it appears in section in Pl. VIII., Figs. 5 and 10, *q*. They are not developed as rod-cells, yet their shape, their position, their innervation, and their relation to the pigment cells seem to indicate that they are to be compared to the accessory portion of the eyes of the other *Cyclosalpæ*. Yet, in spite of the presence of this peculiar group of cells, the eye of *C. dolichosoma-virgula* resembles those of the true *Salpæ* more than it does the eyes of the other *Cyclosalpæ*. If the *Cyclosalpæ* are more primitive than the *Salpæ*, as many structural features seem to indicate, the study of the eye of *C. dolichosoma-virgula* would suggest a transitional stage from one group to the other.

Another resemblance between *Cyclosalpa dolichosoma-virgula* and the true *Salpæ* is seen in the fact that in the former species and in *Salpa runcinata-fusiformis* there is found above the ganglion a peculiar ectodermal invagination which opens dorsally by a relatively narrow mouth, and which probably serves as a protection for the ganglion and eye, no large optic chamber being present in either of these species.

The position of the large eye in the chain salpas is worth noting. In most species the eye projects upwards (*Salpa runcinata-fusiformis*, Fig. 20, Pl. IX.) or forward from the dorsal face of the ganglion (compare Fig. 5, Pl. VIII., which shows a median section of the ganglion and eye of *Cyclosalpa dolichosoma-virgula*). In *Salpa punctata* the eye projects slightly downward from the antero-dorsal surface of the ganglion (Fig. 12, Pl. IX.). In *Thalia democratica-mucronata* the eye projects downward and backward from the antero-ventral surface of the ganglion (Fig. 17, Pl. IX.). These conditions, and the manner in which in *Thalia* the ectoderm is drawn down in front of the ganglion, indicate that the whole ganglion with the eye has rotated forward and downward. Compare Figs. 5, 12 and 17, and note that in

Cyclosalpa dolichosoma-virgula the positions of these parts is the usual one; in *Salpa punctata* the shifting has been slight, about 45°, in *Thalia* the rotation is greatest, 180° or more. The position and arrangement of the nerves in *Thalia* confirms this interpretation.

Remembering the rotation that has occurred in *Salpa punctata*, it seems clear, on comparing Fig. 5, Pl. VIII., and Fig. 12, Pl. IX., that the group of rod-cells lying behind the origin of the optic nerve, in the dorsal part of the ganglion, in *S. punctata* corresponds to the postero-dorsal protuberance from the ganglion in *Cyclosalpa dolichosoma-virgula*, although in the latter species rod-cells are not found in this region. There is great diversity between species in the extent to which groups of rod-cells are developed in the periphery of the ganglion. A similar postero-dorsal protuberance containing no rod-cells is found in *Cyclosalpa pinnata* (Fig. 18, Pl. IX.).

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Explanation of Plates.

a, accessory mass of optic cells; *b*, small cells of latero-ventral outgrowths from ganglion; *b'*, large cells of latero-ventral outgrowths from ganglion; *bl*, blood sinus; *d*, duct of neural gland; *dp*, dorsal pigment cells of large eye; *dr*, dorsally-directed rod-cells of large eye; *e'''*, "accessory portion" of *Cyclosalpa pinnata* eye; *ec*, outline of ectodermal chamber above the ganglion; *ex*, dorsal group of rod-cells in ganglion of *Salpa runcinata-fusiformis* and *Salpa punctata;* in Fig. 17 it indicates a group of optic cells in *Thalia democratica-mucronata* which may be homologous with the cells marked *ex* in the *Salpæ* (cf. Figs. 12 and 20, Plate III.); *ey*, *ey'*, *ey''*, groups of rod-cells in peripheral cellular layer of ganglion of *Cyclosalpa dolichosoma-virgula;* *ez*, a group of optic cells in *Thalia democratica-mucronata* which is apparently homologous with one of the posterior limbs of the horseshoe-shaped eye of the chain form of *Cyclosalpa pinnata;* *g*, ganglion; *gc*, fibrous core of ganglion; *gz*, membrane surrounding ganglion; *h*, neural gland; *hz*, membrane separating gland wall from large cells of latero-ventral outgrowths; *i*, dorsal intermediate cells of large eye; *i'*, ventral intermediate cells of large eye; *l*, peripheral cellular layer of ganglion; *lp*, lateral pigment cells of large eye; *lr*, laterally-directed rod-cells of large eye; *n*, nerve; *nc*, nerve cells; *o*, opening of duct of neural gland into the pharynx; *oc*, optic chamber; *on*, optic nerve; *os*, optic ectodermal sheath; *oz*, optic membrane covering eye; *p'''*, pigment cells of "accessory portion" of *Cyclosalpa pinnata* eye; *pc*, pigment curtain in *Salpa punctata* eye; *pr*, postero-dorsal protuberance on ganglion of *Cyclosalpa dolichosoma-virgula*; *q*, group of spindle-shaped cells in mid-dorsal region of *Cyclosalpa dolichosoma-virgula* eye; *q'*, group of cells posterior to pigment curtain in *Salpa punctata* eye; *r'''*, group of rod-cells in eye of *Cyclosalpa chamissonis*; *se*, supra-neural ectodermal chamber of *Salpa punctata*; *vl*, ventro-lateral outgrowth; *vp*, ventral pigment cells of large eye; *vr*, ventrally-directed rod-cells of large eye; *gw*, thickened portion of gland wall; *x*, nerve which arises from one of the ventro-lateral outgrowths; *y*, outline of optic chamber; *z*, limiting membrane of optic chamber.

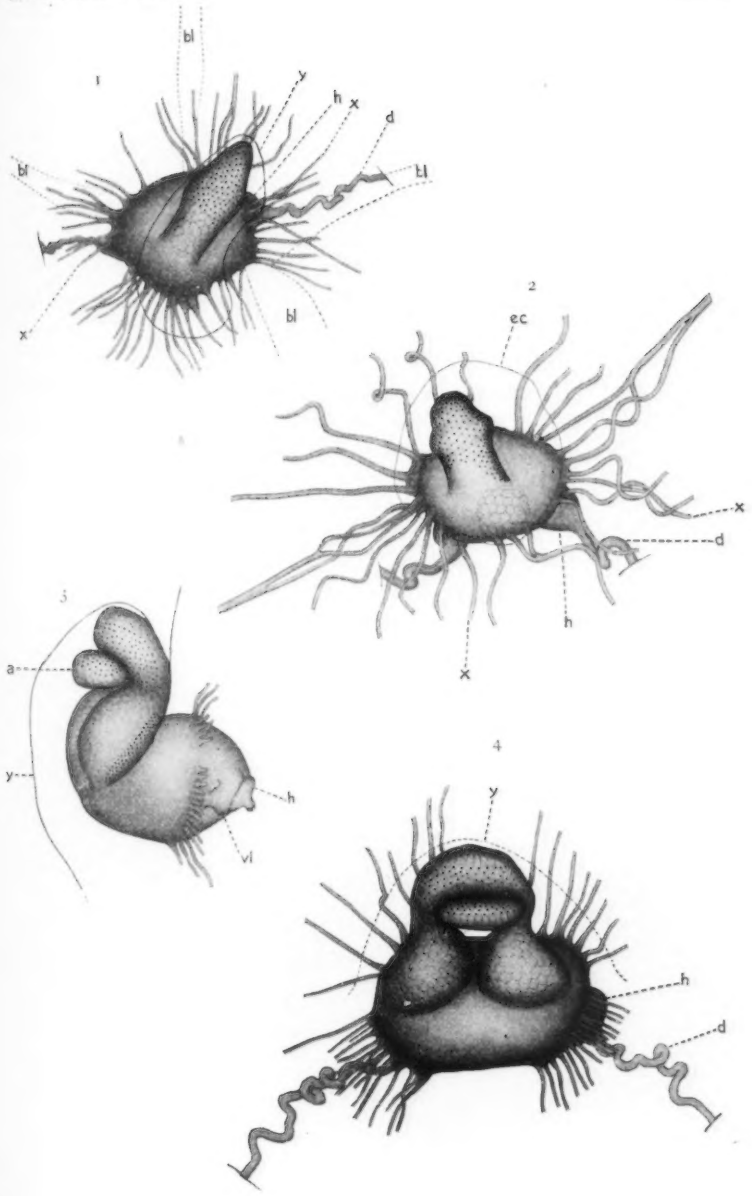
Explanation of Plate VII.

Fig. 1. Dorsal view of eye, ganglion and nerves of *Cyclosalpa dolichosoma-virgula* chain form, showing also neural glands and ducts. The pigment of the eye is represented by heavy stippling. The ends of the rod-cells when visible are indicated by faintly stippled outlines. The outlines of some of the blood sinuses are indicated by dotted lines. The extent of the optic chamber is indicated by a contour-line.

Fig. 2. Dorsal view of the eye, ganglion, nerves, glands and ducts of *Salpa punctata*. The pigment of the eye is represented by heavy stippling. The rod-cells in the dorsal part of the ganglion are indicated by faintly stippled outlines. The rod-cells of the posterior portion of the large eye lie below so thick a layer of nerve fibers that their outlines do not show.

Fig. 3. The eye, ganglion, nerves and gland of the chain form of *Cyclosalpa pinnata* seen from the right side. The heavy stippling indicates pigment. The faintly stippled outlines indicate the contours of the rod-cells. The contour-line indicates the extent of the optic chamber. (Copied from Metcalf.)

Fig. 4. Dorsal view of the same. (Copied from Metcalf.)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Explanation of Plate VIII.

Figs. 5–11. *Cyclosalpa dolichosoma-virgula*, chain form. Compare Fig. 1.

Fig. 5. Longitudinal vertical section of eye and ganglion, compounded from three oblique sections.

Fig. 6. Longitudinal section through the latero-ventral outgrowths from the ganglion and through one neural gland.

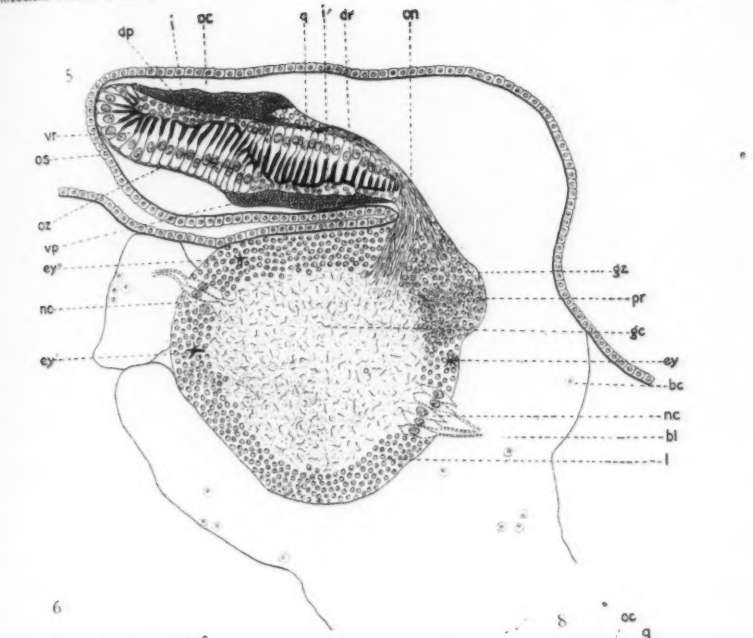
Fig. 7. Cross-section through the eye near the tip.

Fig. 8. Cross-section through middle of eye.

Fig. 9. Cross-section through eye near the base.

Fig. 10. Enlarged drawing of the section of the middle portion of the eye shown in Fig. 5, to show group of spindle-shaped cells, *q*.

Fig. 11. Enlarged drawing of one of the groups of rod-cells in the peripheral cellular layer of the ganglion.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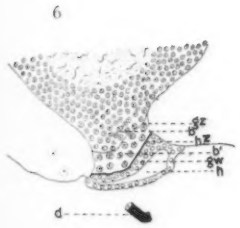
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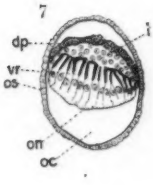
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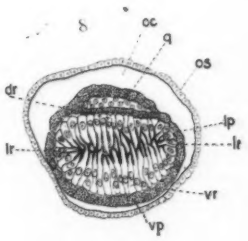
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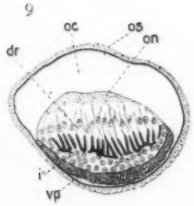
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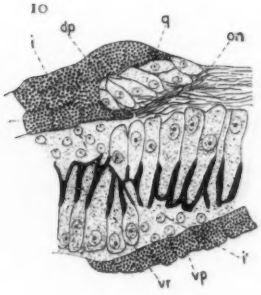
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Explanation of Plate IX.

Figs. 12–16. *Salpa punctata*, chain form. Compare Fig. 2.

Fig. 12. Longitudinal vertical section of eye and ganglion.

Fig. 13. Cross-section through the eye near the tip.

Fig. 14. Cross-section through the middle of the eye.

Fig. 15. Cross-section through the eye near the base. In a section still further back the pigment would appear ventrally as well as laterally.

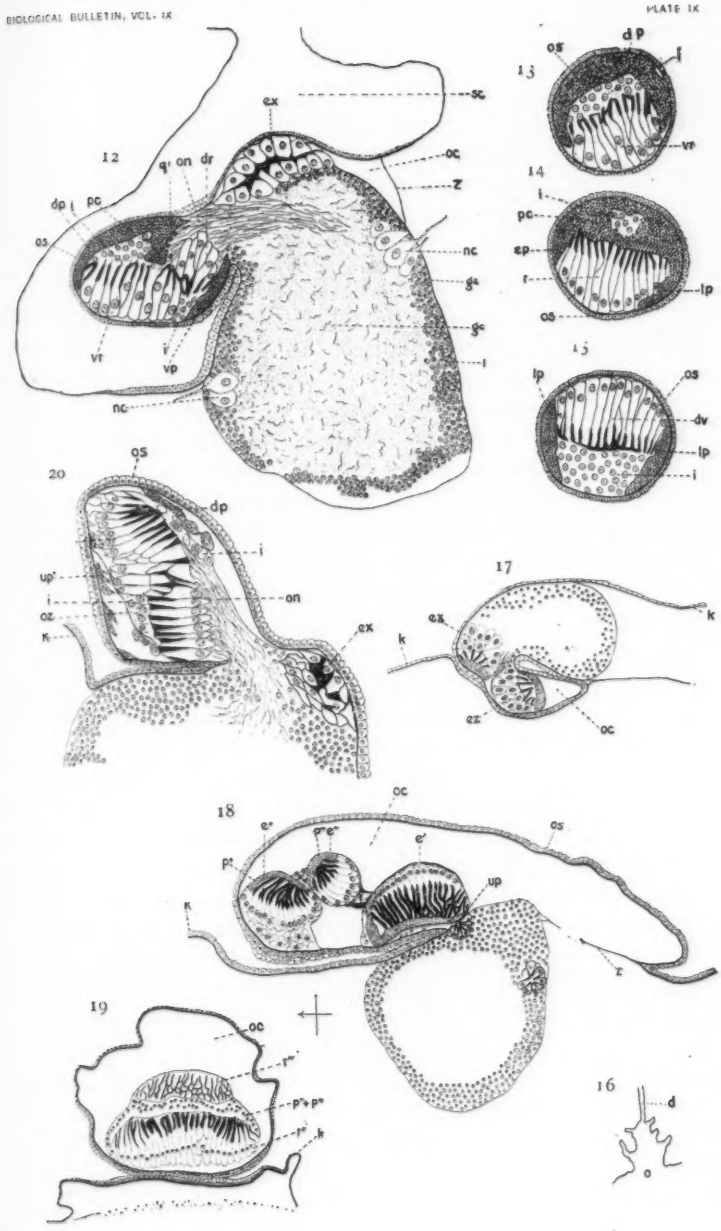
Fig. 16. Horizontal section through the distal end of the duct of one neural gland, showing convolutions at the aperture of the duct.

Fig. 17. Longitudinal vertical section of the eye and ganglion of *Thalia democratica-mucronata*, chain form (copied from Metcalf).

Fig. 18. Longitudinal vertical section of the eye and ganglion of *Cyclosalpa pinnata*, chain form (copied from Metcalf).

Fig. 19. Oblique (horizontal-cross) section through the middle of the eye of *Cyclosalpa chamissonis*, chain form (copied from Metcalf).

Fig. 20. Longitudinal vertical section of the eye and anterior part of the ganglion of *Salpa runcinata-fusiformis*, chain form (copied from Metcalf).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THE EXCRETORY AND CIRCULATORY SYSTEMS OF CRYPTOCHITON STELLERI MIDD.

HAROLD HEATH.

Somewhat over half a century ago Middendorff ('49) published his extensive studies on the anatomy of *Cryptochiton stelleri*, the largest and in some respects the most highly modified of the chitons. In several important details this was a decided contribution to our knowledge of the group and yet the work has not received the attention it has merited chiefly for the reason that the facts are inextricably associated with numerous errors (due to poorly preserved material) and are illustrated by figures often difficult to interpret. It is the purpose of the present paper to explain and to a certain extent correct Middendorff's results and especially to describe some of the more noteworthy features of the excretory and circulatory systems. This latter disvision of the subject may appear unnecessary owing to the extensive chiton studies already published, notably those of Haller ('82), Pelseneer ('99), Thiele ('02), and especially Plate ('97), but as will be noted in the following pages this species differs in several fundamental particulars from any other known form.

The specimens on which Middendorff's studies were based came from the shores of Kamchatka and from that locality this species extends to the southward fully 2,500 miles, the southern limit being approximately Monterey Bay, California. Throughout the greater part of their range, as I know from personal observation, they are confined to the littoral zone, rarely extending into water more than three fathoms in depth. Usually they are more or less concealed among the red algæ (especially *Gigartina exasperata*) upon which they feed and with which they harmonize so closely that they escape the untrained eye. While the largest northern specimens in my possession measure less than 20 cm. (8 inches) in length, some in the vicinity of San Francisco not infrequently are 25 cm. (10 inches) long. The largest specimen I have ever seen measured slightly more than

33 cm. (13 inches) when alive and in a resting condition and weighed seven grams less than two kilograms (4.4 lbs.).

Regarding the breeding habits little may be said at the present time. On three different occasions I have found males shedding their sex products in the latter part of February and at this time the females are distended with eggs. The oviduct is provided with an albumen gland in all essential respects like that of *Ischnochiton magdalenensis* (Heath, '99) and it is reasonable to suppose that like this last-named species *Cryptochiton* lays its eggs imbedded in a gelatinous envelope. In the early part of the summer the young have attained a length of from 10 to 22 mm., and as has been described in a previous paper (Heath, '97) the shells are still exposed. The mantle of these small individuals is usually of a yellow or orange color, exceptionally light green, and is beset with more or less definitely arranged yet scattered groups of crimson spicules characteristic of the adult. As these increase in number the general shade ordinarily changes to a brick red[1] not infrequently blotched with patches of white or purplish-white that usually disappear by the time the animal has become sexually mature.

Middendorff was the first to discover the kidney in the chitons yet it was with some hesitancy that he applied this name to it, as his observations were very incomplete. However, it must be said they are more perfect than some of his critics have supposed. He correctly states that the excretory canals unite in front of the pericardium in "einem geschlossenen Bogen" (p. 73) and he accurately locates the glandular portion, but the fact was never discovered that this latter division is penetrated by two canals, one of which connects with the pericardium while the other opens to the exterior.

The kidney holds the usual position at the sides of the visceral cavity and posseses the form of a very slender U, the free extremities terminating in the reno-pericardial, and external openings, while the opposite rounded end is situated at the level of the anterior margin of the third valve of the shell (Fig. 1).

[1] Middendorff described his specimens as yellowish brown but this was due to the fact that the tufts of bristles had been worn away exposing the mantle to an abnormal degree.

At the extreme lateral border of the percardium and about one fourth its greatest length from its anterior end the relatively large inner opening occurs (Fig. 2, *r*) and almost immediately leads into a flat disc-like cavity closely attached to the ventral pericardial wall. From the inner border of this flattened sac a slender delicate tube, usually almost invisible in preserved material, proceeds forward and becomes continuous with the more dorsal tube of the glandular portion of the kidney proper. This last-named section consists, as in chitons generally, of numerous highly branched lobules which extend ventrally some distance on the inner surface of the foot, dorsally to the valves of the shell and to the head cavity in front.

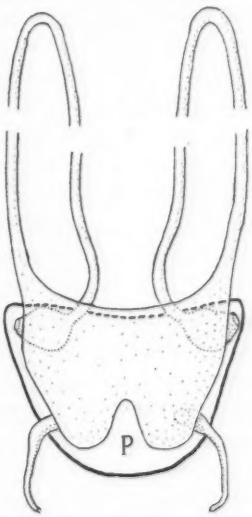


Fig. 1. Diagram illustrating the kidney of *C. stelleri*, ventral view; *p*, pericardium.

In a number of species of chitons the outer limb of the excretory canal expands into a well defined reservoir (Nierensack), which is also supplied with glandular outgrowths. In some cases these reservoirs are of comparatively large size and in a few cases they may extend close to the mid-line, but here, as in every species of chiton hitherto described, one kidney is wholly independent of the other. In *Cryptochiton*, on the other hand, the reservoirs, that are probably distinct in very young specimens, unite in the mid-line and form, as Figs. 1 and 2 show a spacious chamber lying ventral to the anterior third of the pericardial cavity. In specimens one fourth the adult size, and occasionally in individuals of much greater length, there are slight indications of a median partition that may represent the line of fusion of the once independent divisions.

Carefully removing the rectum and opening the ventral wall of this common reservoir the latter will be found to terminate posteriorly in two triangular diverticula, each of which communicates by means of a narrow slit immediately behind the trans-

verse blood sinus (sinus transversus) with a dorsal disk-like cavity in contact with the ventral pericardial wall (Fig. 2). From the antero-lateral border of each of these last-named spaces a slender tube arises and passing outward plunges beneath the afferent branchial sinus and then curving backward gradually approaches the surface of the body and opens through the renal papilla. The position of the external excretory opening is, unlike that of the genital papilla, remarkably constant in position. In one hundred specimens examined on this point the excretory

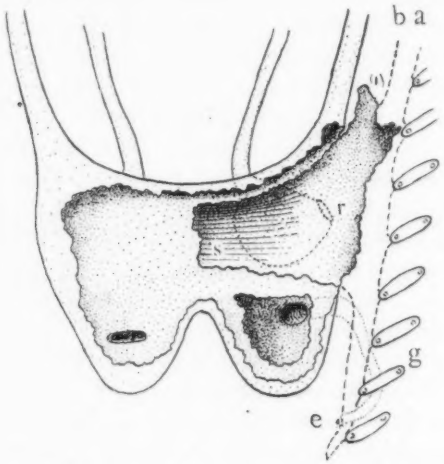


Fig. 2. Posterior part of kidney of *C. stelleri*. The ventral wall has been removed and a portion of the dorsal in order to show the transverse sinus (*s*) and the exit of the ureter; *ba*, branchial artery; *e*, outer kidney opening; *g*, gill; *r*, renal pericardial opening; genital opening opposite eighth gill.

pores were, with a very few slight exceptions, situated opposite the last gill.

Connective tissue cells laden with concrements, such as were described by Brock ('83) for several molluscs, are present in *Cryptochiton* in great abundance. In all specimens, but notably those above 10 cm. in length they form great accumulations in the foot and mantle in the vicinity of the mantle furrow and give the tissue the appearance of having been charged with light yellow sand which cuts with a distinctly gritty sound. Cells of this

character are distributed to a greater or less extent throughout the entire foot and mantle but sooner or later they make their way to the main accumulations by means of amœboid movements and possibly through the agency of the blood stream. In the young of several species of chitons (less than 1 mm. in length) these concrement bearing cells may be seen to originate from the mesenchyme elements that also form the connective tissue, blood and apparently some of the muscle elements. The concrements arise in the form of one or two small refringent granules in each cell and gradually increasing in size and usually in number sooner or later almost completely fill the cell which during this period usually takes up a position near some blood sinus. In the description of the circulatory system attention is called to the fact that a large blood sinus (lateral sinus) with numerous branches penetrates these "Granulazellen" but the relation of the two remains uncertain. Sections not infrequently give the impression that these cells passing into the sinus disintegrate after which the resulting products may be taken up by the kidney but there is no definite assurance that such is the case.

There are only two papers in which a serious attempt has been made to trace the circulation of the blood in the chitons. The first by Middendorff is very incomplete and in many respects incorrect; while the second by Plate is much more detailed yet in certain fundamental particulars, it is not in accord with the results set forth in the following paragraphs.

As we know from the work of Middendorff the heart in this species is essentially the same as in other chitons consisting as it does of a median ventricle and the lateral auricles. This author however made the mistake of claiming that these latter chambers end blindly behind (for they are united in the usual fashion) and that the ventricle gives off numerous small branches in the median ventral line to the rectum and laterally to the mantle edge and last valve of the shell. The supposed openings in this region are merely the depressions marking the attachment of delicate muscle fibers (trabeculæ carneæ) that span the cavity of the ventricle. In the auricles the same depressions appear but as injections clearly show the only blood, besides that from the efferent branchial sinus, that enters these chambers comes from the mantle at

the extreme posterior end of the animal through two small vessels close to the mid-line behind and in certain cases even these openings appear to be absent.

The relations of the aorta to the heart and head cavity and the origin of the genital arteries Middendorff correctly determined but the account of the last-named vessels is somewhat obscure. A careful examination shows that they course between the folds of the inner wall of the gland very much as in *Acanthopleura echinata* (Plate) and after branching repeatedly (and often not dichotomously) become lost among the developing sex products.

Concerning the course of the blood from the gonad I have nothing to add to Plate's account of *Acanthopleura echinata* except that in *Cryptochiton* a relatively large quantity leaves from the neighborhood of the gonoducts and enters the extensive sheets of kidney tissue adjoining the front end of the pericardium. From here very little blood appears to go into the visceral cavity but penetrating the spongy tissue of what may be termed the lateral space (Seitenlückenraum, Midd.; Lateralkammer, Plate) is poured into the lateral sinus (Seitenarterie, Midd.).

The dorsal arteries, lateral vessels between the II–V valves, supply some of the shell muscles in the fashion accurately described by Plate. Owing probably to the greater size of the mantle in *Cryptochiton* the intersegmental arteries are more numerous and of larger size than in other species of chitons. They arise sometimes singly (dividing almost at once) though usually in pairs from the dorsal side of the aorta between each valve and as well defined vessels may be traced for considerable distances. Usually these vessels do not extend far into a neighboring "segment" but in all of the specimens carefully examined on this point the vessels between segment V–VI are invariably of large size and extending latterly and posteriorly supply the greater part of the hinder portions of the mantle. The blood returning from the pallium pours into the lateral sinus.

Middendorff (p. 70) states that these small vessels (called by him Mantelarterien) appear to end blindly in the leathery substance of the mantle and on the other hand to open into the lateral sinus and the branchial artery. Plate also makes the statement that in *Acanthopleura echinata* most if not all of the

blood from the mantle passes into both the branchial artery and the branchial vein. In *Cryptochiton* the vessels from the mantle are of comparatively large size and may be followed without difficulty into the lateral sinus. Injections of the branchial artery and the branchial vein give no indication that they receive any blood directly from the mantle.

The marginal vessel (Randgefäss) imbedded in the mantle to the outside of the mantle furrow, a position corresponding to the lateral fold (Lateralfalte) of other chitons, is but a system of irregular sinuses supplied by vessels from the intersegmental artery. The blood returning from it passes, together with other blood from the mantle, into the lateral sinus.

In *Cryptochiton* the course of the blood leaving the head cavity is considerably different from that in any other chiton hitherto described. According to several investigators the blood leaves the head sinus by the following vessels: the visceral artery, the lateral sinus of the foot, vessels passing into the snout (Mundscheibe) and by means of canals surrounding the pedal and pallial nerves. In *Cryptochiton* no blood makes its direct exit by either the pedal or pallial neural sinuses and the course of the blood passing into the snout is considerably different from that described by Plate for *Acanthopleura echinata*, and furthermore there is a pair of pallial arteries.

The head cavity in the chitons is a comparatively narrow space surrounding the buccal mass and separated from the visceral cavity by a septum. Among the more important arteries leading out from it are two of large size which spring from the postero-lateral borders of the snout (Fig. 3). Almost immediately each divides, the inner branch, the pedal sinus, passing into the foot, the outer canal, the pallial artery, following along the mantle cavity in close proximity to the pallial or lateral nerve. The pallial sinus shortly after its origin gives off a branch which ramifies throughout the tissue of the proboscis supplying with its fellow all of the region behind the mouth. It is also in communication with an extensive sinus encircling the snout as will be described presently. By means of injections each pallial sinus may be followed into the region of the reproductive opening where, after having gradually diminished in size, it vanishes com-

pletely. Delicate branches supply the tissue of the immediate region, some going to the lateral nerve another portion to the tissue of the lateral space and it is possible that another quantity though very small may enter the adjacent regions of the mantle.

Middendorff (pp. 69, 70) speaks incorrectly of two pairs of vessels which arise from the head cavity and passing in close proximity to the mantle furrow open into the sinus transversus (arcus arteriosus). In *Cryptochiton* the only vessel which

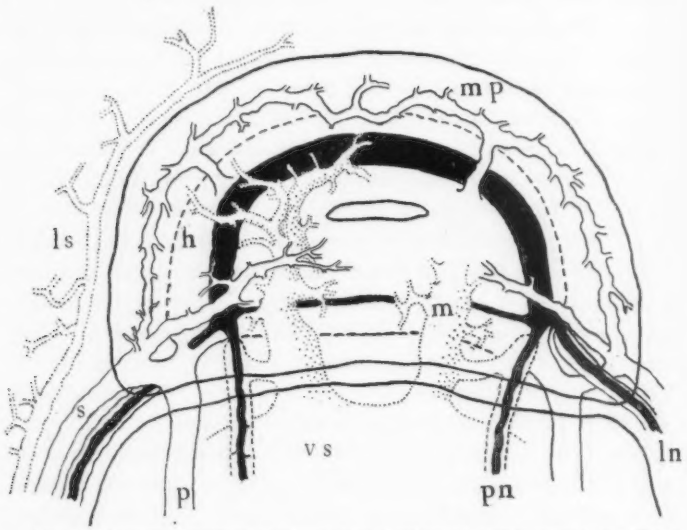


Fig. 3. Diagram illustrating the circulation of snout and certain vessels leaving the head cavity in *C. stelleri*. *h*, head cavity; *ln*, lateral nerve sinus; *ls*, lateral sinus; *m*, median proboscis protractor; *mp*, marginal proboscis protractor; *p*, pedal sinus; *pn*, pedal nerve sinus; *s*, pallial sinus: *vs*, visceral cavity; nervous system, black.

holds such a position posteriorly is the lateral sinus and this as is well known has no connection whatever with the head cavity, and on the other hand the pallial artery connects with the head cavity but does not communicate with the transverse sinus.

Heretofore it has been held that the sinuses surrounding the pallial cords conduct a considerable amount of blood from the head cavity into the mantle but in not less than 50 specimens of *Cryptochiton* injections from the head sinus failed to penetrate

these canals and dissections show that at or near the point of union of the pallial and pedal cords a connective tissue and muscular septum (Fig. 3) completely separates the nerve sinuses from the head cavity. However a short distance behind this point and throughout the greater part of the succeeding portions of the mantle furrow there are frequent communications between the pallial and nerve sinus so that this latter space and adjacent regions are probably filled in life with comparatively pure blood.

In *Cryptochiton* another part of the blood passing out of the head cavity enters the proboscis or snout chiefly by means of two large vessels located on each side of the mid-line about level with the mouth (Fig. 3). Almost immediately these break up into two or three branches that pass outward to the margin of the proboscis where they become continuous with an extensive system of spaces forming a semicircular vessel (*mp*) coextensive with the margin of the snout. From this marginal sinus smaller vessels pass inward and furnish fresh blood to the proboscis tissue, while another supply of arterial blood, much smaller in amount, passes into the snout through sinuses surrounding the nerves. As the figures indicate no part of the blood from any of these sources passes into the tissue of the foot, at least not in any appreciable quantity, but is conveyed along the region of the mantle furrow by means of the relatively large pallial sinus leaving the proboscis at its outer posterior angle, or it may be passed into the visceral cavity as described presently.

Middendorff figures (*f'*, Fig. 2, Pl. VIII.) a small curved canal which surrounds the snout anteriorly and states (p. 72) that it lies between the external and internal oral sphincter. Owing to the fact that in one specimen he was able to force the injection mass from the branchial artery into this vessel he considered it to be the anterior union of the branchial arteries but as these latter vessels certainly do not extend much farther forward than the most anterior gill and have no direct connection with the proboscis, I am of the opinion that the connections as described are incorrect and that the marginal vessel is the same one shown in Fig. 3.

Not only are the margins of the proboscis capable of considerable expansion owing to the influence of an abundant blood sup-

ply but as may be seen in living specimens the more central portions bounding the mouth are likewise subject to great distension. This is produced by a system of vessels present also in *Acanthopleura echinata* according to Plate though their connections are considerably different. From the extreme anterior and ventral part of the visceral cavity they arise as two relatively very wide canals one on either side of the mid-line. Coursing almost directly forward they enter the proboscis and giving off vessels into the more superficial portions encircle the mouth almost to the mid-line in front. Upon the contraction of some of the more anterior pedal muscles the visceral cavity is decreased in volume and a portion of the blood coursing through spaces in the region of the stomach is forced into the proboscis resulting within a short time in a marked increase in size and roundness of its central portions. In recently killed specimens pressure on the front part of the foot produces this phenomenon.

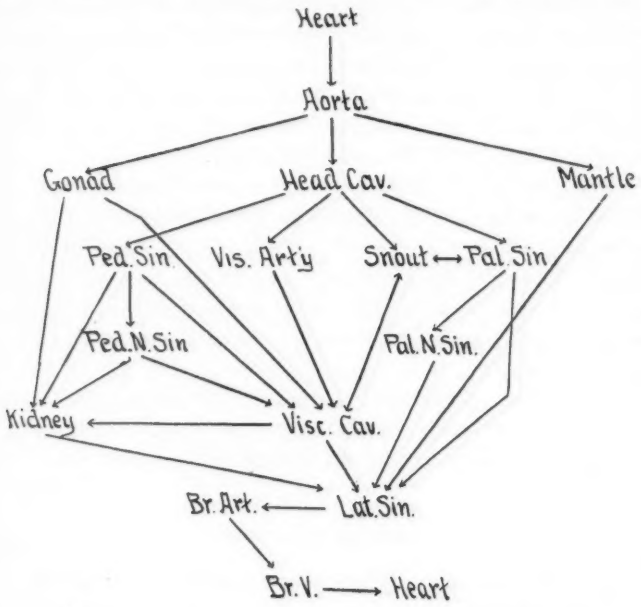
From injections it appears very probable that a considerable portion of the venous blood from the snout, when this is not protruded, passes into the visceral cavity by means of these protractor sinuses. The marginal portions of the proboscis and possibly the more central regions also are relieved of a portion, seemingly small, of their blood supply by means of several highly branched vessels emptying into the lateral sinus external to the snout.

In *Acanthopleura echinata* according to Plate the foot is provided with a median sinus which anteriorly connects with two vessels surrounding the mouth and corresponding to the median proboscis protractors of *Cryptochiton*. Were these latter vessels in *Cryptochiton* to be separated with a portion of the visceral cavity and fashioned into a median pedal sinus the relations would be essentially the same as in the above-named species. However the course of the blood leaving the median sinus is not by the usual exit from the visceral cavity for Plate states that it passes into the branchial artery.

The blood enters the foot by the two pedal sinuses, whose relations are already known, and leaves by two routes—into the visceral cavity or along the transverse pedal muscles to the kidney. As has been shown the contraction of some of the anterior pedal muscles may drive a quantity of blood into the proboscis, thus

causing its protrusion, and it is reasonable to suppose that according to the state of contraction of the pedal muscles generally the blood pressure of the visceral cavity may vary, causing a flow of blood from the foot into the cavity or in a reverse direction. Injections show that in dead or flaccid animals the line of least resistance is from the pedal sinuses laterally into the kidney.

As with the pedal nerve sinus a well-defined septum destroys any communication with the head cavity (Fig. 3) but throughout its entire course it is in frequent communication with the pedal



sinus which supplies it and the adjacent regions with aerated blood.

In all of the specimens carefully examined a connection exists between the pedal nerve sinus and the bases of the median protractor sinus (*m*) so that blood may pass from one to the other. Throughout its greater extent the pedal nerve sinus receives its blood from the pedal sinus and it is difficult to understand this peculiar anterior connection unless it is some device to aid in the protraction of the anterior margin of the foot.

Middendorff correctly describes the visceral artery which springs from the head cavity and surrounding the radula sac supplies the alimentary canal and liver. The blood passing from the capillaries pours into the visceral cavity and joining that from the foot, proboscis and gonad proceeds through the meshes of the kidney to enter the lateral sinus.

The lateral sinus in *Cryptochiton* is a relatively large canal surrounded by connective tissue and muscle fibers in whose wide meshes are vast numbers of "granulazellen." Anteriorily it arises near the mid-line in the furrow surrounding the snout from which it receives a few small vessels while numerous others of large size drain the blood from the mantle. Coursing backward and constantly increasing in size it continues to receive vessels from the mantle and finally in the vicinity of the reproductive opening it unites with the branchial artery.

The accompanying diagram will serve to illustrate the course taken by the blood in passing through the body, at least it represents the main canals. It will be seen that the well-developed lateral sinus plays an important part, acting as a collector for the blood from all parts of the body and possibly its surrounding walls serve to remove certain wastes before being passed to the branchial artery.

The relations of the branchial artery and the branchial vein to the gills and the connections of the latter vessel to the heart are already well known and require no further comment.

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ON THE HETERONEREIS STAGE OF NEREIS KOBIENSIS McINTOSH.[1]

AARON L. TREADWELL.

Among a collection of polychætous annelids made by the U. S. F. C. steamer Albatross in the Hawaiian Islands in 1902, and sent me for study, were large numbers of a heteronereid which by means of the general character of the head, the presence of a peculiar hook-shaped seta in the anterior parapodia, and especially from the form of the paragnathi, I have identified as the above-mentioned species.

Externally the bodies of both male and female individuals show very clearly the distinction between "modified" posterior, and "unmodified" anterior somites characteristic of this sexual phase of *Nereis*, the modifications following in general the usual direction, *i. e.*, a broadening and flattening of all parapodial lobes and a replacement of the ordinary form of seta by one with a very broad, flat, terminal joint. A constant external sex difference is found in the male in the dorsal cirri of somites 2–7. Beginning with somite 2 the dorsal cirrus is larger than in the female, and on successive somites as far as the seventh there is a gradual increase in the size of this organ, until on the seventh it is very prominent, composed of a thick, cylindrical basal portion; with a broad flattened tip, ending in an acute point. This condition is similar to that of the form described by Verrill under the name of *Nectonereis*.[1] On the eighth somite there is an abrupt change to the ordinary form of cirrus.

Internally, extensive modifications appear. On a surface view of a mature female, one sees that the whole body, anterior, "unmodified" as well as posterior "modified" region is crowded with eggs, seeming not to be isolated in somites, but packed together in a continuous cavity. That the transverse septæ are actually

[1] Published by permission of Hon. George M. Bowers, United States Commissioner of Fisheries.

[2] Verrill, A. E., "Invertebrates of Vineyard Sound," Bull. U. S. F. C., p. 591, 1872.

lost is indicated further by the fact that by gently pressing on the surface of the body wall, eggs may be moved from one somite to another or through several somites.

Dissection and the study of transverse sections confirm these conclusions. By the loss of the transverse septæ the whole interior of the body has been transformed into a continuous thin-walled sac, filled with ova from one end to the other. No trace of ovaries could be found, and, owing to the poor preservation of the material nothing definite can be said concerning the histology of the internal organs, but there is every indication of a considerable amount of degeneration. While the pharynx retains

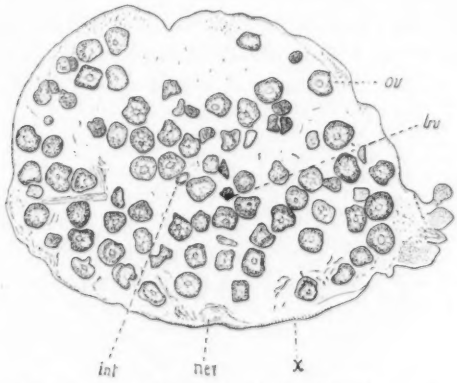

FIG. 1. Section through anterior region of body of female. × 24. *ov*, ova; *bv*, blood vessel; *int*, intestine; *ner*, ventral nerve cord.

its normal condition the intestine appears on gross dissection as a mere thread, and anteriorly, the thin transparent body wall, lacking most of its usual muscle layers, is very noticeable.

Fig. 1 is from a transverse section through the anterior end of the body, thus through the "unmodified" region. The body wall, with the magnification employed, appears as a mere line, with the thin layer of longitudinal muscle fibers showing as rows of dots just inside it. These muscle fibers are more or less broken away from the rest of the wall, but I cannot tell how much of this is due to actual degeneration, and how much to defective preservation and faulty microtome technique. A small

break in the dorsal wall is apparently due to the latter. The intestine, *int.*, Fig. 1, shows in the section as a minute tube, in whose walls no definite cellular structure could be seen. In many sections I could find no trace of the intestine. While this may possibly be due to the technique, I do not think that they could have dropped out of so many sections, and believe that there was an actual disappearance. Such a condition with respect to the intestine is not unusual among annelids at the breeding season.

Ventrally is the section of the nerve cord, and on the right a part of the parapodium is seen. The parapodial muscle bands are very feebly developed.

As shown in Fig. 1 large numbers of ova are present in the body cavity. These fill the whole cavity, extending forward into the head, so that, as is shown in Fig. 5 a section through the

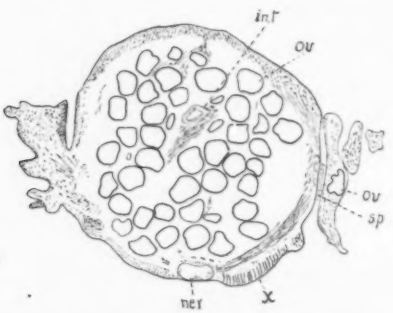

FIG. 2. Section through posterior region of same individual as in Fig. 1. × 24. *sp*, spermatozoa adhering to surface, other letters as before.

eye shows at the same time an ovum, contained in a cavity between the muscles of the head. That this condition is usual can be seen by careful surface examination of the entire annelid. The ova appear to have a very dense outer layer with vacuolated interior containing a nucleus. As it is impossible to tell how much of this apparent structure is an artefact, I have represented it only in a very diagrammatic fashion in Fig. 1, while in Figs. 2 and 5 I have drawn merely the outlines of the ova.

Scattered among the ova are many sections of connective tissue

and blood vessels, *bv*, Fig. 1, indicating that loose strands of this tissue, carrying vessels, still persist.

In the posterior portion of the body, the so-called "modified" region, similar conditions hold with respect to the septæ and intestine, but the body wall is very noticeably thicker, its muscle layers being very well developed. This is easily seen by reference to Fig. 2 which is a section through a posterior somite of the annelid drawn in Fig. 1. The longitudinal band of muscle fibers is much more strongly developed, and the oblique fibers, especially those connected with the parapodia are vastly stronger than those in the anterior somites. The difference in thickness of the body wall is especially well seen in a comparison of Fig. 3, an enlarged camera drawing of the body wall at *X* in Fig. 1, with Fig. 4, a similar drawing of *X* of Fig. 2. The dermal layer is

FIG. 3. Detail of ventral wall at *X* in Fig. 1. × 280. *lm*, longitudinal muscle fibers seen in section; *ep*, epidermis.

FIG. 4. Detail of ventral wall taken from the point 4 in Fig. 2. × 280. *cut*, cuticle.

much thicker, and the longitudinal band is relatively enormously developed. It will be understood, of course, that the thickness of the muscle band is indicated by the entire diameter of the figure. Owing to the irregular arrangement of the fibers no one of them extended through the bundle in a straight enough fashion to appear entire in a single section. It is evident further that the arrangement in Fig. 4 is more nearly a normal one than in Fig. 3, so that the condition of the latter must be regarded as due to degeneration.

On either side Fig. 2 shows a section of a parapodium, that on the right containing an ovum in its cavity.

Similar conditions hold in the case of the male heteronereid. The anterior "unmodified" portion of the body is more or less

degenerate as regards its body wall, muscles and intestine, while the posterior "modified" region shows at least no degeneration of its muscle structure. I am inclined to believe there has been a hypertrophy of these organs, but non-sexual individuals are not available for comparison. Spermatozoa are found all through the body, from the prostomium backward. At no place, however, does the intestine appear as degenerate as in the female. This is especially true of the posterior region, where the intestine is large, filling nearly as much of the cœlom as it does in other annelids.

FIG. 5. Section through head of same individual. × 24. *e*, eye; *tent*, tentacle.

If the loss of the intestine has arisen in the female through mechanical reasons, connected in any way with the accumulation of large numbers of ova around it, it is easy to see why the loss should be greater in the female than in the male. Though present in large numbers, the spermatozoa would on account of their small size, occupy much less room and exert much less pressure on surrounding organs, than would the ova.

Many individuals contained no sex cells, apparently having discharged them into the water. That the sea water must have contained considerable numbers of sex cells is shown by the fact that numbers of spermatozoa are found in the sections, adhering to the body of the female. Fig. 2 represents a number of sperm adhering to the outside of a lobe of the parapodium.

As shown above, no sex organs were found. It would be of a good deal of interest to know whether they were originally present throughout the body, or are confined to the posterior "modified" end. The latter condition is much the more probable. Apparently in this annelid there is, in the heteronereis phase, a considerable degeneration of the anterior "unmodified" region, the degeneration involving the internal organs and parapodial muscles, though not especially involving the external organs. Into this thin-walled sack thus formed pass the sex-cells, which are now carried in it (as well as in the posterior portions of the body) while the posterior parapodia retain their normal muscular development and function as the main loco-

motor organs of the animal. Whether the animal goes to pieces after the sex cells are thrown off, or whether it subsequently regains its normal condition, cannot be determined by the material at my disposal.

VASSAR COLLEGE, March 3, 1905.

THE REPAIR AND REBUILDING OF THE LARVAL CASE OF PLATYPHYLAX DESIGNATUS WALK. (PHRYGANID).

WM. S. MARSHALL AND C. T. VORHIES.

The larvæ of *Platyphylax designatus*[1] are found abundantly in a group of springs in the vicinity of Madison, and, being easily obtained at any time during the year, have been collected for work in the laboratory. Experimenting with a few larvæ which we kept in small aquaria, we noticed that those which had been removed from their cases constructed new ones and also that injuries to the cases, by removal of some part, were generally repaired in a short time by the larvæ. This led us to carry on a number of experiments to determine how far injured shells would be repaired by the larvæ, and, if certain parts of the cases were more frequently and readily rebuilt than others.

The springs in which these Phryganid larvæ live remain open all the year; this was a great advantage to us in that material could be readily obtained throughout the winter. The water in the springs is very cold during the summer as well as in the winter months and the larvæ, being accustomed to very cold water, would not live, during the summer, in aquaria kept in the laboratory or out of doors. We found it advisable to carry on our experiments during cold weather, at which time it was easy to keep the aquaria in cool places, bringing them into the laboratory only when our specimens were examined. In the autumn and in the spring, just before or after freezing began or ceased, we found the most convenient times for our work, as at these periods of the year the aquaria could be kept on a ledge outside of the laboratory window.

The cases of the larvæ of *Platyphylax designatus* are constructed, with few exceptions, from the sand which covers the bottom of the springs in which they live. Irregularities in the size of the sand grains used are sometimes observed, but there

[1] The identification of the species was made by Mr. C. Betten.

is, in general, a regular arrangement; the posterior, oldest, part of the case is made entirely of small grains, the anterior, newest, part of the shell of much larger and more irregular grains; between the two ends there is a gradual increase in the size of the sand grains used. As the larvæ increase in size the shell is made wider and larger grains are used in its construction; after building is completed, or nearly so, there are often two or three very large pieces placed near the anterior end. When the larvæ begin to prepare for pupation nearly all the pieces added are of greater size than any of the others, and, finally, when the case is closed, the anterior fourth or third is nearly covered with these large grains, which are so marked and constant that by their presence one can easily tell a larval from a pupal case. Often just before pupation a larva would close the posterior end of its case by the addition of a single large sand grain over the opening at this end. After closing the shell for pupation a small opening was always left at the anterior end. This was found between two of the large sand grains, the space between which was filled with a layer of the secretion from the spinning glands, and in this the opening was found.

Two different kinds of plant seeds were sometimes used in the construction of the cases and these, with the rare use of pieces of snail shells, composed with the sand grains, the materials of construction. In many instances when the seeds had been used in building a case but one or two of them were used; cases were, however, often found in which were a dozen or more of the seeds and often the two kinds were found in the same case. We had several larvæ with these cases living for a few days in the laboratory where the water remained warm and this sometimes resulted in the germination of the seeds. When this happened the larvæ presented a curious appearance, their cases having a few young plants, one quarter to one half an inch in length, growing on them.

The experiments we made were upon the construction of new cases by larvæ from which the original cases had been removed, and upon the repair of cases after certain parts had been cut out. To facilitate the work and make it possible for us to tell exactly how much new shell had been added, we placed in our aquaria,

not the brownish sand from the springs in which the larvæ lived, but crushed red sandstone. When a larva rebuilt the part of the shell we had removed it was easy to see just how much new material had been added.

The construction of a new case is easily observed by removing larvæ from their cases and placing them in aquaria at the bottom of which is some sand. The larvæ appear at first much distressed and wander around in the aquaria attacking each other, if a number are in the same dish, but never, so far as we have observed, injuring one another. From the fact that the caseless larvæ always attack each other it is much better to place but a single one in an aquarium. Many will remain for one or two days without attempting the construction of a new case while others will begin building within an hour, or in a few instances, in even less time. When a larva begins the construction of a new case it first, with its mandibles, gathers a few of the larger sand grains and cements them together with the secretion from the spinning glands. This done, the larva gathers other grains from a distance carrying them in its mandibles to the pile already formed, and, as each is brought, it is cemented to the others. In this way, in the course of from two to four hours, a large pile of sand grains is made, each grain of which is cemented to the others, forming all together a loose mass nearly as large as the larva itself. It is now somewhat surprising to notice that the larva, in beginning to construct its shell, does not, apparently, use this pile at all but begins its case at one side and adjacent to it, and there, from smaller grains than those in the pile, the new case is built. The first process in the actual construction we did not see but the larva soon has a narrow band of case around its body and to this, at the anterior end, new grains are continually added. As the new case increases in length, the diameter is slightly enlarged although the new cases do not show as much difference in width at the two ends as did the old shell. When the case has reached the necessary length the larva turns in it and begins to pull down the posterior margin, finally making the opening at this end smaller than the diameter of the shell. The larva again assumes its original position and finally builds a slight dorsal hood at the anterior end. During the construction

of the case the original pile of sand grains has been attached to its posterior end and may be dragged around by the larva; it is however finally cut off and remains at the bottom of the aquarium. Whether or not the construction is similar in the natural habitat of the larvæ we do not know.

Cases cut Longitudinally from End to End.

Without removing the larvæ the cases were cut with scissors from end to end and on different surfaces. It was necessary to cut the cases slowly avoiding the largest sand grains and taking care not to injure the larvæ.

1. The cases of three larvæ were cut, one dorsally, one ventrally, and the third along the lateral surface. The following day the laterally cut one had glued the cut surfaces together in the anterior region; the other two had not been repaired.

2. The experiment was repeated with three more larvæ and at the end of twenty-four hours all had glued, anteriorly, the cut edges together for one third the distance.

3. The case was cut ventrally in one larva and on the following day it had been repaired the same as above.

4. A larva with case cut dorsally gave the same result.

5. On the twenty-sixth of the month a case was cut dorsally and the larva removed from it; the following day it had returned to its case and cemented the edges at the anterior end for about one quarter of the distance. Twenty-eighth, the larva had cut out a notch at the anterior end, at terminus of original cut, and built in with new sand. Twenty-ninth, had added a few sand grains to posterior edge of shell. First of following month, had added a band, two grains wide, at the anterior margin.

6. On the twenty-fifth a case was cut dorsally which by the following day had been repaired at the anterior end for about one third its length. Twenty-eighth, case cut again in the same plate. Twenty-ninth, no repair. Thirtieth, edges glued a little at anterior end and two rows of sand grains added to this margin. Thirty-first, same. First, cut again. Second, glued anterior third of distance and added from four to five more rows of sand at this end; cut again in same place. Third, again glued at

anterior end and more sand added, making a total of 2.5 mm. Fourth, cut again. Fifth, had cemented at anterior part; cut again. Sixth, cemented as before; cut again. Seventh, eighth, ninth and tenth, cementing and cutting repeated each day. Eleventh, cut edges at anterior end cemented as before and a little more new sand added to this end.

7. Six cases were cut, two dorsally, two ventrally, and two laterally. The time during which these experiments were carried on was at least two weeks in each instance, giving sufficient time for full action on the part of each larva.

Of the two cut dorsally one ignored the cut entirely but built on a ring of new sand at the anterior end after eight days had elapsed. The other at once cemented the cut edges for a distance of 2 mm. at the anterior end, then, for a period of nine days, did nothing further. Repetition of the cutting had no other effect than to cause, within a short time, the larva to glue the edges for a short distance at the anterior end.

The two cut ventrally took much more notice of the injury to the case than the preceding. One of these closed the cut half way down from the anterior end and at the end of two days had completely glued the cut edges together. Repeated cutting caused, with one exception, a partial repair within twenty-four hours. After five such repetitions an additional day was allowed for repair, at the end of which time, the cut edges were completely glued. The second had at the end of twenty-four hours cemented the cut at the anterior end for a distance of 2 mm.; then for eight days it did no further repairing. The cut was then reopened and at the end of twenty-four hours was again partially closed. The next day the larva had turned in its case and was working at the posterior end and on the following day had completely cemented the cut edges.

The first of the two laterally cut specimens would always close the cut for about one third the distance at the anterior end, and once, when allowed four days, completely repaired the injury. Twice a new ring was added at the anterior end instead of the usual repairing. The second of these larvæ did nothing for four days. It then closed the cut for a distance of 2 mm. at the anterior end and for four additional days made no further repair.

The cut was now well opened daily for seven days. On each of the first two days a distance of 1 mm. at the anterior end was repaired, on the third and on the fourth days 2 mm., and on the fifth and sixth days a distance of 4 mm. was repaired. The repairing on the sixth day was very poorly and loosely done and, no repair being made on the next, the seventh day, the experiment was abandoned.

The cutting of the cases from end to end causes no inconvenience to the larva and no exposure of its body. In all examples cut it was difficult to see, without a fairly close examination, that a cut had been made; the firmness of the case causes the cut edges to either touch along their entire distance or, in many instances, to slightly overlap. The repair was always begun at the anterior end, the larvæ paying but slight attention to the opposite end although, in a few cases, this was also repaired and it may be, that if the cut cases had been in every instance left unmolested for several days the ultimate result would, in all experiments, have been a completely repaired case. The distance of the repair at the anterior end represents the distance which can be readily reached by the larva without turning in its case.

Notches cut in Case at Anterior End.

These notches were cut in from the anterior margin of the case and on its different surfaces; the cuts were in general equal in depth to one fourth or one fifth the length of the shell. While not always of the same size and shape the piece removed was generally very nearly 3 mm. in length and 2 mm. in width at its base, the base of the triangular piece cut out being always along the anterior margin of the case.

1. Twenty-ninth, lateral notch cut; in two days this was half filled with new sand grains; the next six days no change. In thirteen days the notch had been completely filled in and a few grains added to the anterior edge.

2. Dorsal notch cut, was completely repaired in two days.

3. Dorsal notch cut and partially repaired by following day; in building a small opening was left by the larva at the apex of the notch and this was never repaired. In two days the anterior margin was built even to rest of case.

4. Dorsal notch cut in case and it was completely filled the following day; this was repeated seven more times and each time the notch was filled in twenty-four hours; the ninth time the repair took two days. The larvæ now added a narrow band to the anterior edge of the case; this was removed and rebuilt three times in as many days.

5. Dorsal notch cut and repaired seven times daily with one exception, in which the period of repair extended for two days.

6. Ventral notch cut six times and repaired five times, each at end of twenty-four hours, and once after interval of two days.

7. Two cases, in each of which a dorsal notch was cut, were repaired by the following day.

8. Dorsal notch cut but not repaired for three days.

9. Lateral notch cut and repaired in three days.

10. Two cases notched, one dorsal and one ventral, were both repaired in twenty-four hours, but in each instance a small opening was left at apex of notch which was never repaired.

Any portion of the case which has been removed from the anterior part, provided the piece removed includes part of the margin, was repaired in a short time. If the piece removed was taken from the shell at some place away from the margin, repair is neglected and the opening allowed to remain. In two of the experiments recorded it will be noted that in filling in the notch a small part was left open and this was never repaired. Some larvæ not recorded had holes cut in their shells and after a lapse of several days the openings were still unrepaired. A larva can easily fill in a notch 2 × 3 mm. in twenty-four hours.

Case Cut Transversely into Two Pieces.

In the following experiments the cases were cut transversely into two pieces, which were as nearly equal as we could make them without an actual measurement. The larvæ were then replaced in the aquaria with both pieces of the case still on their bodies.

1. On the first of the month a case was cut and one piece, 6 mm. in length removed. Second, at 8 a. m., the new part added to old piece left on the larva was nearly equal to the part removed, and at 4:30 p. m. it was of the same length. Third,

the new part was now 8 mm. long, or 2 mm. longer than the part removed, and it was also somewhat wider. Fourth, new piece 10 mm. in length. Seventh, larva had added 2 mm. more and closed the shell for pupation.

2. Fourteenth, case 11 mm. in length cut and both pieces left on larva. Fifteenth, the larva had thrown off the posterior piece and built 2.5 mm. to anterior end of piece (anterior) which it had retained; total length 7 mm. Sixteenth, length of shell had been increased to 11 mm., the original length.

3. Fourteenth, case 10 mm. long was cut and both pieces left on the larva. Fifteenth, the posterior piece had been thrown off and 3.5 mm. built on to anterior end of remaining piece. Sixteenth, enough had been added to make entire length of case 10 mm.; equal to the original.

4. Second, a case 11 mm. long was cut and on the following day the posterior half had been thrown off and 4.5 mm. added to anterior end of the piece retained by the larva. During the day the larva reversed itself in the case and turned in the posterior edge; total length 9 mm. Fourth, removed the new anterior part which the larva had built. Fifth, had again added 4.5 mm. to old part of case. Eleventh, abandoned old shell and built a new one which, by the fourteenth, was 9 mm. long.

5. Second, a shell 9 mm. in length was cut and by the following day enough had been added to the anterior piece of the old case to make it 10 mm. long. Fourth, cut between old (posterior) and new (anterior) parts. Fifth, had thrown off the old (posterior) piece and added 3 mm. to other part. Seventh, case had total length of 9 mm. Fourteenth, total length 10 mm.

6. Fourteenth, cut a shell 10 mm. long; the same day the larva threw off the posterior piece and added 2 mm. to the anterior end of the piece. Sixteenth, length of new part only 3 mm. and on the next day had increased to 3.5 mm.; total length of case 9 mm.

7. Thirty-first, case 11 mm. in length was cut and on the following day the posterior piece had been thrown off and 4.5 mm. added to anterior end of the piece retained by the larva. Third, case was cut between the new and the old parts. Fourth, old part (posterior piece) had been thrown off and enough added to

other piece to make total length of shell 10 mm. Sixth, the posterior end was turned, total length of shell 10.5 mm.

8. Second, shell 9 mm. long was cut: on the following day the posterior piece had been thrown off and 3 mm. added to anterior end of remaining part. Fourth, posterior end had been turned; 3 mm. of the new shell removed. Fifth, 1.5 mm. added. Seventh, total length of shell now 7 mm.; following day the larva was dead.

9. A shell 12 mm. long was cut and on the following day the two pieces were glued together and a few new sand grains added to the anterior end.

10. A shell 12 mm. in length was cut and on the following day the posterior part had been thrown off and 5 mm. added to anterior end of remaining part. After an interval of one more day 7.5 mm. of new shell had been added; total length 12.5 mm.

11. Fourteenth, shell 12 mm. long cut. Fifteenth, posterior part had been thrown off and 2 mm. added to anterior end of remaining part. Sixteenth, 5 mm. more added. Seventeenth, 0.5 mm. added: total length 9.5 mm.

12. Thirty-first, case 11 mm. long was cut. First, posterior part thrown off and 4.5 mm. added to anterior end of other part. Second, no change. Third, cut again at boundary of old and new parts. Fourth, the posterior piece again thrown off and enough added to make total length of case 10 mm. Eighth, posterior end turned, total length of case 10.5 mm.

In but one of these experiments did the larva glue the two cut margins together and when this had been done the case was nearly as good as before cutting, except its greater flexibility and a slight lack of strength. In every other experiment the larvæ threw off one of the pieces, always the posterior, and built on to the anterior margin of the remaining (anterior) piece. The construction of the new part of the case was rapid, averaging nearly 4 mm. the first twenty-four hours; to this more material was added until the case had entirely or nearly reached the original length. One half of the case is not long enough to entirely conceal the body of the larva and the first work is to increase the length of the case sufficiently to do so, after which what we might call the finishing touches, *i. e.*, turning in the posterior margin and building of a hood at the anterior end are finished.

REMOVAL OF PART, OR WHOLE, OF POSTERIOR END.

1. Thirty-first, cut off the posterior end of a case and by the following day the larva had turned in the edge; cut again. Third, had slightly turned the edge. Fourth, cut 2.5 mm. from posterior end. Fifth, had added 5 mm. to anterior end. Eighth, 2.5 mm. more added. Tenth, posterior edge turned.

2. Small piece cut from the posterior end and by following day the edge had been turned and four grains of sand added at this end; case but slightly smaller than before cutting. Following day the larva was dead.

3. Second, cut piece 4.5 mm. long from posterior end of a case which was 11.5 mm. in length. Third, added 3 mm. to anterior end. Fourth. 1 mm. more had been added. Seventh, 2.5 mm. more added and anterior end closed. Eighth, both ends of case closed and it was glued to a leaf for pupation.

4. Second, cut piece 3.5 mm. from posterior end of a case. Third, 1.5 mm. had been added to anterior end. Fourth, 0.5 mm. more added. Tenth, new part now 3 mm. in length, posterior end not yet turned.

5. Ninth, cut 4 mm. from posterior end of a case and by the following day 3.5 mm. had been added to the anterior end. Fourteenth, a few sand grains added to the posterior end; case closed for pupation.

These few experiments on the posterior end show that the larvæ are loath to work at this end of the case and the additions to the shell were made at the anterior end. As the cases are normally turned in at the posterior end most injuries to this end will result in the larvæ ultimately reversing themselves in the case to re-turn the margin. We watched the larva turn from a reversed to a normal position, the whole process occupying about four minutes. At first the posterior end of the body is protruded from the anterior opening; the head is then bent forward and soon appears at the opening. Next, by a seemingly very strenuous effort, the body is withdrawn into the case and the larva assumes its normal position. During the process the second pair of legs are thrown up into a position dorsal to the thorax and pointing backward. The larva takes a short rest during the reversing process. That the effort is an extremely

hard one is evinced by the fact that we have, during our experiments, found larvæ which have been "stuck" in turning and died without being able to completely turn.

Notches Cut at the Posterior End.

1. Four cases were cut, two dorsal and two ventral. The two ventrally and one dorsally notched case were closed the following day, but without the use of sand, the secretion from the spinning glands being used and the cut edges partly drawn together. The fourth specimen did nothing on the first two days; the third day, and, thereafter each day for five days, new grains were added to the anterior end until a new part 4 mm. in length had been built.

Two experiments were made by cutting, day after day, a small piece from the posterior end of the case, thus forcing the larva to add continually to its case if it desired to keep it long enough for its body. It was found that either daily or every two or three days a small portion would be added; if daily, a smaller amount than if the larva waited one or two days before making the repair. No tendency to exhaustion of the silk was noticeable during the three weeks or more that the experiments lasted.

No. 1 built a total length of new case 17.5 mm. in twenty-one days; average 0.83 mm. daily.

No. 2 built a total length of 19.5 mm. in twenty-three days; average 0.84 mm. daily.

With several larvæ the experiment was tried of driving them out of their cases and then placing both larva and case in the same dish to ascertain if they would return to old case. Two larvæ were placed in each dish, a large and a small one, to see whether a larva would occupy a case built by another; with this arrangement there could of course be but one change, that of the smaller larva into the case of the larger. When new cases were constructed these were destroyed leaving only the original ones.

1. Did not return to old case. Built a total length of 32 mm. of new case in the first four days. Rested a day and then added 6 mm. more in two following days. Total length of new case built in seven days 38 mm. Average 5.42 mm. per day.

2. Reëntered case at the anterior end, the regular method, but in reversing stuck fast and died.

3. Returned to old case five times out of seven trials. Built 12.5 mm. in eleven days. Average 1.1 mm. per day.

4. Out of eight trials returned to its old case twice and to that of larger larva once. Built five new cases in thirteen days with a total length of 42 mm. Average 3.23 mm. per day.

5. Did not return to its old case. Out of eight trials lasting thirteen days a new case was built each time but one. Daily average 4 mm.

6. In six trials built a new case each time then occupied case of larger larva twice, the last time adding a new part of smaller diameter to the shell. In twelve days built 42 mm. of new case. Average 3.5 mm. per day.

7. Out of six trials returned to old case five times, building only 5 mm. of new material. Went without case for two days during which time the old case was occupied by another larva. Daily average of new case construction only 0.38 mm.

8. In eight trials a larva returned once to its own case, three times to that of a larger larva, and four times built new cases; 23 mm. of new cases were built in fourteen days. Daily average 1.64 mm.

9. Did not return at all to old case but built, in fourteen days, a total of 65 mm. Average daily construction 4.64 mm.

10. November 7, took a larva out of its case and it immediately crawled in again at the anterior end. Eighth, had assumed proper position in case and added new ring of sand grains at anterior end. Removed again from case but it reëntered as before. On the ninth, tenth and eleventh, the larva was removed from its case but each time time reëntered and added a little new material to the anterior end, 3 mm. in all. Twelfth, removed from case but did not again return and not until the sixteenth was a new case constructed, at which time it was 10 mm. long. Seventeenth, case 15 mm. in length. Eighteenth, case 16.5 mm. long; removed larva from it. Nineteenth, had constructed a new case which was 11 mm. long. Twenty-first, new case had 2 mm. more added; removed the larva. Twenty-second, another new case 7 mm. long had been built; again removed the larva.

Twenty-third, the larva had returned to its original case. During the six days of its greatest activity in building the larva had constructed 36.5 mm. of new case, a daily average of 6.08 mm. The daily average for the entire experiment was 2.25 mm.

ZOÖLOGICAL LABORATORY, UNIVERSITY OF WISCONSIN,
April, 1905.

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A SHORT REMARK UPON W. H. LEWIS' "EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE EYE IN AMPHIBIA."[1]

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In the above-mentioned article, published in Volume III. of the *American Journal of Anatomy* (1904), W. H. Lewis has given us the results of a large number of ingenious experiments on frog-larvæ intended to determine the possible correlations between the development of the optic vesicle and that of the lens.

These experiments have, in the first place, offered a new and, as it seems to me, very convincing proof that there is indeed, as first maintained by Herbst and Spemann, a distinct correlation in the development of these two organs, namely in such a way "*that in normal development the lens is dependent for its origin on the contact influence or stimulus of the optic vesicle on the ectoderm.*"

Furthermore Lewis has, by transplanting the optic vesicle to another part of the larval body, demonstrated "*that the optic vesicle can stimulate a lens to form from various portions of the ectoderm and even from the ectoderm from the abdomen of another species of frog. . . .*" From this fact he has drawn the following conclusion: "There is no predetermined area of the ectoderm which must be stimulated in order that a lens may arise. On the contrary various portions of the skin when stimulated by the contact of the optic vesicle may and do give rise to a lens. Not only will a lens arise from various places on the skin as a result of the contact of the optic vesicle of the same animal, but the optic vesicle of one species may cause a lens to rise from the ectoderm of another species of frog." This latter result of Lewis' experi-

[1] Lewis, W. H., "Experimental studies on the development of the eye in Amphibia. I., On the origin of the lens, Rana palustris," *American Journ. of Anatomy*, III., 1904.

ments is of very great interest, in so far as it gives a new evidence of the *equipotential nature of the ectoderm elements* during a certain early period of development. However in further discussing his grafting-experiments and some other observations made in connection with them Lewis is also led to a criticism of a theory recently advocated by me, *namely that the lens has to be considered as a modified primitive sense organ.* As this criticism seems to be based upon a misconception of my article on this subject[1] I feel induced to write the following remarks in explanation.

Lewis says on page 535 of his paper: Schaper's theory "will not hold in view of the fact that the ectoderm, taken from over the abdomen of *R. sylvatica* and grafted on over the optic vesicle of *R. palustris* (see experiment XII., 51), did not possess at the time of operation the primitive sense organs and yet it gave rise to a lens. Again it seems unlikely that in several instances in which I have been able to bring about lens formation from a strange ectoderm that the optic vesicle should have in each case come in contact with one of these sense organs. And again in such experiments as IV., in which the optic vesicle has never been in contact with the ectoderm which normally gives rise to a lens there is no trace of a rudimentary lens such as Schaper pictures." From this remark it is obvious that Lewis holds, that according to my opinion the lens should arise in the course of *ontogenetic* development from a *preformed* primitive sense organ of the skin.

In refutation of this, the following must be said. In the first place I have to state that nowhere in my above cited paper have I spoken of a *preformed* primitive sense organ ("Sinnesknospe") as being the *ontogenetic* forerunner of a lens. What I have maintained is, that the structure which arises from the stimulated part of the ectoderm, to form the lens, shows in its development and its primitive histological features a striking resemblance to a primitive sense organ of the skin, and that this resemblance becomes still more striking when, as in my experiments, by atypical development the lens-anlage is retained within the ectoderm. The

[1] Schaper, A., "Ueber einige Fälle atypischer Linsenentwirklung unter abnormen Bedingungen. Ein Beitrag zur Phylogenie der Linse und zur Mechanik ihrer Entwicklung," *Anatom. Anzeiger*, XXIV., 1904.

evidence of this similitude is, as I think, fully demonstrated by the figures 9, 10 and 11 in my paper. Again, I have by no means considered the anlage of the lens within the ectoderm as a regular (*i. e.*, functional) sense organ ("Hautsinnesorgan"), as I have especially emphasized in the statement: Es "liegt uns selbstverständlich der Gedanke fern, unsere Lentoide functionell auf gleiche Stufe mit einer Sinnesknopse stellen zu wollen, . . .". I have always spoken of a *phylogenetic*, never of an ontogenetic deduction of the lens from a primitive sense organ. Finally I have expressed the opinion that the formation of "Sinnesknospen" is one of the most primitive tendencies of all ectodermal elements and that in any part of the ectoderm a bud-organ may arise under adequate stimulus. This being admitted, the conclusion seems to me justified that also the contact stimulus of the optic vesicle upon the ectoderm may awaken these most primitive properties of the ectodermal cells and result in the formation of a structure *morphologically* homologous with a primitive sense organ which in the course of phylogenetic development has gradually entered a new path of differentiation.

Taking all this into consideration, *the absence of preformed primitive sense organs in that part of the skin where the optic vesicle comes in contact with it and where in consequence the lens is formed is by no means an objection against my theory.* When my theory holds that the lens, from a morphological point of view, has to be considered as an ancient sense organ, which arose, it is true, *originally* from a primitive sense organ of the skin ("*Placoden-Organ*" *of Kupffer*), it does not of course imply the necessity that during *ontogenetic* development of later animal forms we should still be able to see the lens evolve *directly* from such an organ. Just as the mode of development of so many other organs, that of the lens also may have undergone, in the course of phylogenetic progress, considerable modifications until the original process of differentiation becomes more or less concealed. Now, if an organ, as in the case of the lens, *still* exhibits in its early ontogenetic features some distinct reminiscences of ancestral characteristics, we are all the more justified in drawing conclusions from such phenomenon in regard to the ancestry of the organ in question.

Hence, my observations upon the development of the amphibian lens (especially under abnormal conditions) having assured me of a close resemblance of this process to the differentiation of a primitive sense organ, I have advocated this fact as a new evidence, in addition to many others, of the originally sensory nature of the crystalline lens.

NOTES ON THE NORTH AMERICAN SPECIES OF BRANCHINECTA AND THEIR HABITATS.

H. L. SHANTZ.

STUDIES FROM THE ZOÖLOGICAL LABORATORY, THE UNIVERSITY OF NEBRASKA, UNDER THE DIRECTION OF HENRY B. WARD, No. 62.

Three species of *Branchinecta* have been recorded for North America: *B. paludosa* (Müller) Verrill has been reported from Labrador and Greenland. This is an arctic species and is found also in northern Europe and in Siberia. *B. coloradensis* Packard and *B. Lindahli* Packard have been found in the United States.

Of the last two species *B. coloradensis* is the better known. It has been found in Colorado, where it occurs in great numbers, and is recorded for several different localities, all of which, however, are near the center of the state. The original collections were made "near Twin Lakes Creek" south of Leadville, at an elevation of about 3,800 m. (Packard, 1874: 621). Later it was taken near Gray's Peak not far from Georgetown at an altitude of about 3,658 m. and at Weston's Pass southeast of Leadville at an elevation of 3,557 m. (Packard, 1883 : 339). After an interval of 21 years it has been recorded recently from Dead Lake in the Pike's Peak region at an elevation of 3,340 m. (Ward, 1904 : 139).

B. Lindahli was collected first in a pool at Wallace, Kansas, by Professor Joshua Lindahl, and described and named by Packard (1874 : 339–340). Since then, Lafler and Pearse (1898) reported "five or six" specimens from De Witt, Nebraska, and Beardsley (1902: 43) records "one female with eggs" from a temporary pool near Greeley, Colorado. Accordingly this species occurs at a much lower elevation than *B. coloradensis*.

During the summer of 1904 the writer collected 122 adults and many larvæ of *B. coloradensis* from Dead Lake, in the Pike's Peak region. Nine specimens were taken from the same Lake on August 12, 1903.

On October 23, 1904, Professor Aven Nelson collected 16 specimens of *B. Lindahli* at Laramie Hills near Laramie, Wyoming, at an

elevation of 2,317 m. These specimens were found "in an eroded limestone bowl in a canyon always dry except after showers. The bowl will hold a half dozen tubs of water and is rarely entirely dry." This collection was sent to Doctor Ward and was identified by the writer. It contained 10 males and 6 females. This record greatly extends not only the known geographical distribution of this species, but also its vertical distribution. It now belongs with *B. coloradensis* and *B. paludosa* to the short list (Zschokke, 1900: 188) of Phyllopods already recorded in alpine

Species.	*B. coloradensis.*					*B. Lindahli.*					*B. paludosa.*		
	Number of Specimens Measured.	Average.	Maximum.	Minimum.	Measurements by Packard.	Number of Specimens Measured.	Average.	Maximum.	Minimum.	Measurements by Packard.	*B. artica* Verrill.[1]	*B. granulatica* Verrill.[1]	Measurements by Packard.
Length of male.....	40	19.6	23	16	18	10	13.6	17.5	11.2	8	20	17	15–19[2]
Length of female.....	45	18.3	20.5	16	17	6	12.8	15.5	10	15	20		12–18
Second antenna of male	40	5.33	9	3.5	7	10	3.87	5	2.26	3			4– 5
First division of second antenna.....	40	3.344	6	2.1	4	10	2.23	2.92	1.29	1.5	1.66	2.87	2.5–3
Second division of second antenna	40	1.986	3	1.2	3	10	1.64	2.11	.975	1.5	1.29	2.24	1.5–2
Eye of male	40	.715	.869	.650		10	.428	.537	.325		.66		
Eye of female	31	.607	.685	.571		6	.321	.373	.276				
Egg.....	25	.328	.373	.308		4	.189	.195	.162				
Length of ovisac	45	6.17	8	4	8	6	3.71	5	2	4–5	6.2		4– 5
Caudal appendage of male + setæ	34	1.88	2.27	1.46		10	1.92	2.6	1.62		1.96	1.62	
Caudal appendage of male	35	1.13	1.46	.812	1	10	1.32	1.62	.975	1	.96	.86	1–1.5
Caudal appendage of female + setæ	6	1.80	1.99	1.19		6	1.95	2.27	1.42				
Caudal appendage of female.....	10	1.143	1.4	.65		6	1.115	1.46	.853	2			

[1] Verrill originally thought these distinct species; his measurements are given in these columns.
[2] Sars places the maximum limit at 23 mm. Measurements are all in millimeters.

situations. This material was carefully measured and compared with the material of *B. coloradensis* and also with the measurements and descriptions of *B. paludosa* as given by Verrill (1870), Packard (1883) and Sars (1896). The results of these measurements are given in the above table:

It is clear from the measurements that *B. coloradensis* and *B.*

paludosa are of about the same size, while *B. Lindahli* is somewhat smaller. The body of *B. coloradensis* is not only larger but more robust than that of *B. Lindahli*. The latter is much more "fairy-like" in appearance, and specimens in formalin are perfectly white or transparent. The color of *B. coloradensis* is much more variable. It has not changed in formalin and varies from creamy white to salmon. In the material collected July 29, 1904, were 26 males and 56 females; the males were light and the females were salmon without a single exception. But of the 14 males and 26 females collected July 12, 1904, there were several exceptions to this color distinction.

Perhaps the most noticeable difference between *B. Lindahli* and *B. coloradensis* is in the size and shape of the eye. (Pl. I.; 13, 14, 15, 16). Packard (1883 : 338) describes the eyes of *B. coloradensis* as "rather larger" than those of *B. paludosa*, and the eyes of *B. Lindahli* as "rather large." A reference to the table shows that the eyes of *B. coloradensis* are much larger than those of *B. Lindahli*, the ratio derived from the average measurements being for the males 1.67 to 1 and for the females 1.89 to 1. It must be understood that, since the writer has only 6 females of *B. Lindahli* the ratio for these may not express as nearly the average as does the ratio for the males. Verrill (1870 : 245) gives 0.66 mm. as the measurement of the eye of the male of *B. paludosa*. This almost equals the smallest measurements of *B. coloradensis*, but is considerably larger than the largest measurements of *B. Lindahli*. In *B. Lindahli* the ocular globe is developed but slightly better on the anterior side than on the posterior side. In *B. coloradensis*, however, the anterior side is developed much more than the posterior, or it may be said that the eye is bent forward abruptly at the place of union between the ocular globe and the peduncle. When viewed from above, these eyes are very distinct from those of *B. Lindahli* and can be distinguished at a glance. Although the eyes of the female are somewhat smaller in each case than those of the males, they are of the same form. Judging from Sars' description (1896 : 44; Pl. VI., Fig. 9; Pl. VII., Figs. 1 and 2) the eyes of *B. paludosa* are more like those of *B. Lindahli* than like those of *B. coloradensis*.

The second antennæ, or claspers, of the males differ rather markedly in these three species. *B. paludosa* is distinct because of the toothed inner margin of the first division, and of the gradually tapering second division which ends in a bluntly rounded point. In both *B. coloradensis* and *B. Lindahli* the distal ends of the second antennæ are turned in rather abruptly (Pl. X., 7, 8; Pl. XI., 23, 24). *B. Lindahli* has, as a rule, shorter claspers than *B. coloradensis*. But this cannot always be depended upon, for the measurements show that it is not an uncommon thing to find *B. Lindahli* with second antennæ longer than those of *B. coloradensis*, even though the body length of the latter exceeds by several millimeters that of the former. There is, however, a considerable difference in the average length of the claspers of these two species. The average measurement of 40 specimens of *B. coloradensis* is 5.33 mm., varying from 3.5 mm. to 9 mm.; while the average of 10 specimens of *B. Lindahli* is 3.87 mm., varying from 2.26 mm. to 5 mm.; the ratio between the averages of *B. coloradensis* and of *B. Lindahli* is 1.37 to 1. Only one individual of *B. coloradensis* had claspers 9 mm. long, and this is probably exceptional, since the next longest measured only 7.2 mm.

The basal division of the claspers of *B. Lindahli* is of about the same thickness as that of *B. coloradensis*. It is somewhat shorter, however, the ratio being 1. to 1.49. The outer margin of the first division of the clasper of *B. Lindahli* has a number of very delicate sensory hairs. Near the base on the inner side there is a raised portion bearing teeth (Pl. X., 7*ta*). Teeth are totally lacking as we approach the distal end. This division of the claspers of *B. coloradensis* is notably different. The toothed area is about half way between the ends on the inner side (Pl. X., 8*ta*). Near the base there is a prominent tubercle of considerable length. This tubercle is characteristic of full grown males. It is not plainly seen unless the clasper is bent forward, when it stands out prominently on the inner side. It is often a third as long as the width of the basal division (Pl. X., 8*t*; Pl. XI., 23).

The second division of the clasper is about the same thickness in the two species. The length is somewhat less in *B. Lindahli* than in *B. coloradensis*. In each species the inturned portion varies greatly in outline, depending on the point of view (Pl. X., 7,

8, 9, 10, 11, 12; Pl. XI., 23, 24). Viewed from the front or back, the tips of *B. Lindahli* are much more gradually pointed than those of *B. coloradensis* (Pl. X., 7, 9, 11), but when viewed from above or below, the reverse is true. This is due to the fact that the already flattened second division of *B. Lindahli* is still more flattened on the outer or lower side as the inturned tip is approached, and this gives the lower part a truncate or slightly convex appearance (Pl. X., 7, 10, 11; Pl. XI., 24). In *B. coloradensis*, instead of the lower part of the tips being flattened, there is developed a prominent ridge and this, together with a less prominent ridge on the upper side, gives the tip a very blunt appearance when viewed from the front (Pl. X., 8, 12; Pl. XI., 23). The tip in *B. coloradensis* is flattened on the anterior and posterior sides, while the tip in *B. Lindahli* is flattened dorso-ventrally.

In *B. paludosa* the second division of the clasper tapers rather gradually to the blunt end, which is not inturned as in the other two species under consideration. The ratio between the length of the second joint and the first is for *B. coloradensis*, 1 to 1.68; for *B. Lindahli* 1 to 1.36; and for *B. paludosa* 1 to 1.28.

In young specimens of *B. coloradensis* the tubercle of the basal joint is often absent. It is also not uncommon to find such specimens with the second division ending rather bluntly, and not inturned. When in this condition, the second division resembles the corresponding division of *B. paludosa*.

The eggs of *B. paludosa* and *B. Lindahli* are of the same size (Packard, 1883 : 340). Those of *B. coloradensis* are much larger, the ratio being 1.74 to 1.

The average length of the ovisac of *B. coloradensis* was 6.17 mm., varying from 4 mm. to 8 mm.; that of *B. Lindahli* was 3.71 mm., varying from 2 mm. to 5 mm., the ratio between the two species being 1.66 to 1. The ovisac of *B. Lindahli* is thicker in proportion to its length, and often contains 50 or more eggs arranged in from three to five rows, while the ovisac of *B. coloradensis* seldom contains as many as 30 eggs, and these generally arranged in from one to three rows (Pl. XI., 17, 18).

The caudal appendages of *B. Lindahli* are much longer in proportion to their breadth than those of *B. coloradensis* (Pl. XI., 19,

20, 21, 22). They are practically identical with the illustration of the caudal appendages of *B. paludosa* as given by Sars (1896 Pl. VI., 10).

Packard's original description of *B. Lindahli* (1883 : 339) is the only one known to the writer. For this description Packard had only the collection made at Wallace, Kansas, by Professor Lindahl. This collection contained 10 females and only 1 male. To judge from the description this male was an exceptionally small one and evidently not a typical specimen. It therefore seems best to give the more important specific characters of the male of this species :

Body long and slender, from 8 mm. to 17.5 mm. in length, less robust than that of *B. coloradensis ;* second antennæ long and powerful, reaching to the base of the fifth or sixth foot, 2.2 mm. to 5 mm. in length, somewhat shorter than those of *B. coloradensis ;* first division 1.2 mm. to 2.9 mm. in length, thick and provided on the outer margin with delicate sensory hairs, an elevated toothed area near the base on the inner side ; shorter than the first division of *B. coloradensis*, of about the same thickness but lacking the prominent tubercle at the base ; second division curved, shorter than the first, ratio to the first 1 to 1.36, the outer surface flattened and meeting the arched inner surface in two prominent angles. When viewed from the front or back, less than half as thick as the basal division. The flattened outer surface bends forward to the prominently inturned tip, which is more flattened below than above. This dorso-ventral flattening of the inturned portion causes it to appear very blunt when viewed from above or below, but to appear rather gradually pointed when viewed from the front or back. Eyes rather large, 0.325 mm. to 0.537 mm. in diameter, the ocular globe but slightly better developed anteriorly than posteriorly, and not much larger than the peduncle : much smaller than the eye of *B. coloradensis* — ratio 1 to 1.67 ; caudal appendages narrow-lanceolate, length plus setæ 1.62 mm. to 2.6 mm.; length of appendage 0.975 mm. to 1.62 mm.; longer in proportion to the breadth than those of *B. coloradensis*.

This species can be distinguished easily from *B. coloradensis* by the smaller eye and egg, by the difference in the shape of the

eye, and by the greater number of eggs in the ovisac; by the absence of the basal tubercle on the second attennæ of the male, by the difference in the tips of the second attennæ, and by the longer and more slender caudal appendages.

It can be distinguished from *B. paludosa* by the absence of the teeth on the inner margin of the basal division, and by the inturned distal end of the second division of the second antenna.

Of the 82 females of *B. coloradensis* only two had both caudal appendages perfect, and only 6 of the 164 single caudal appendages were perfect. Forty males of *B. coloradensis* had only 11 imperfect appendages. No imperfect appendages were found on *B. Lindahli.*

A careful examination of the larvæ of *B. coloradensis* showed only perfect caudal appendages. From this it seems practically certain that the appendages of the adults were originally perfect. The tips of the appendages were first to disappear, and the appendages were then gradually shortened until only a small portion, or nothing at all was left (Pl. XII., 25 to 33). In all but a comparatively few, this change seemed to be a normal process. There was no sign of outward injury, but all the appearance of a gradual removal or absorption of the living substance. This appeared to be the same process by which the large swimming second antennæ of the larvæ are reduced to the much smaller and more simple second antennæ of the adult female.

A number of the appendages of both males and females showed parts of the appendage blackened by disease (Pl. XII., 29, 34 to 38), but this could not be mistaken for the other change. This blackening appeared in only a comparatively few of the appendages, and the number of males and females thus affected was about equal.

The caudal appendages of *B. coloradensis* first make their appearance as little knobs at the end of the common unsegmented body mass (Pl. X., 1, 2). The point of the knob is pushed out to form a stout, short, curved projection which will ultimately develop into the end or one of the end bristles of the mature appendage. By the time twelve body segments have developed, another small point is formed outside of the first bristle (Pl. X., 3). When all the abdominal segments can be seen, two more

points have developed, one on either side (Pl. X., 4). These bristles now develop alternately until all are formed; the constriction between the appendage and the abdomen does not appear until the appendage is almost mature (Pl. X., 5, 6).

B. coloradensis was collected in Dead Lake, a small body of water in the Pike's Peak region. This lake has already been described in some detail by Ward (1904: 131, 135, Pl. XXIX., XXIV., XXV). Without visible outlet or inlet, it lies on the divide between Ruxton Creek and Beaver Creek at an altitude of about 3,350 m. (11,000 feet U. S. Topographic Map). On the southeast side is the ridge leading to the summit of Bald Mountain. This ridge is covered with a dense coniferous forest, shading the lake during the morning. The lake measures about 100 m. by 75 m. and does not exceed 2 m. in depth. The forest barely touches the southeast shore. The remainder of the shore is a mountain meadow, with a few shrubs here and there, and numerous large boulders lying in and about the lake. The bottom of the lake is of blue clay, probably derived from the decomposition of the granite, which is the only rock of this region. It has the appearance of an old lake, one about to disappear (Ward, 1904: 132). There is very little change of level, and the lake is not known to have been dry. For the last 14 years during which time the writer has repeatedly visited this place, conditions have not changed in any marked degree. During the summer of 1904 a sawmill and air pump were operated on the shore, without affecting the life in any marked degree. The water is clear, pure and very slightly alkaline. On July 12, 1904, one drop of dilute H_2SO_4 in which 1 cc. = 50.954 mg., was sufficient to neutralize 100 cc. of lake water, with methyl orange used as an indicator.

The earliest collections were made on May 20, 1904, the day when the ice broke, and while the southeast side was still partially covered with ice. The temperature of the water was 4°.7 C. in the warmest places, and varied to almost 0° C. in the coldest. Many larvæ had appeared, among which were those of *B. coloradensis*. These varied in stages of development from those in which only the antennæ, mandibular legs, and unsegmented body could be seen, to those in which all the segments had been formed.

On June, 4, the ice had all disappeared except at the south-east edge, and the warmest temperature was 6°.2 C. In this collection the larvæ were well developed, but none of those taken had transformed the swimming antennæ. They were larvæ of the last stage (Sars, 1896 : 55, Pl. VI., 5, 6). At the time of the next collection, June 17, the water temperature was 12°.2 C. and none of these forms were taken in the Birge net. While the mature animal was never captured in this way the larvæ of the last stage were easily taken in the net, so that, these forms were probably mature at this time.

The mature animals were extremely abundant July 12, with a surface temperature of 13°.6 C., and were equally abundant July 29, when the temperature was the same. At this time there was only 0°.2 C. difference between the surface and bottom temperatures. On August 12, with a surface temperature of 15.°6 C. the *Branchinecta* had disappeared. After a diligent search, one was seen, but although the search was continued no others were found. The bottom collections made at this time were filled with decomposing fragments of *Branchinecta.*

Ward (1904 : 139) recorded this form for July 13, 1903, when the surface temperature was 14°.4 C., and they were collected by the writer on August 13 of the same year.

From the above, it is clear that segmentation and most of the embryonic development must have taken place under the ice. the writer has no records of the temperature of the water before the ice broke, but this temperature according to Zchokke (1900 : 45) probably did not exceed 2°.2 C. By the time the temperature had risen to 6°.2 C. the larvæ were in the last stage of their development, and at 13°.6 C. the animals had become fully mature. They had disappeared when the temperature had risen to 15°.6 C. This was the warmest temperature recorded for this Lake. The mature forms were never abundant in the open water. They collected about the rocks and near the shore where they could be seen in great numbers swimming on their backs.

On August 12, 1903, as well as on July 12 and 29, 1904, the setæ of the legs and caudal appendages bore numbers of a green alga *Characium ambiguum* Hern. On July 29, these algæ were so numerous that many of the *Branchinecta* could swim only

with slow labored movements, and this may have contributed to the sudden disappearance of the phyllopods.

This lake has no vertebrate fauna of any kind. The most common species besides the one here considered is *Diaptomus shoshone* Forbes (Ward, 1904 : 140) which is a very large form, of a pure deep red color, and very abundant. *Daphnia pulex* De Geer (Ward, 1904 : 149) and *Daphnia longispina* Müller are also quite abundant. The bottom fauna is especially rich in insect larvæ and Turbellaria. The flora is entirely algal, chiefly Conjugatæ, which are relatively abundant. The Schizophyceæ are well represented ; *Merismopedia glauca* Naeg., *Gomphosphæra aponica* Kuetz., *Anacystis pulverens* (Wood) Wolle, and a number of *Oscillatoria* and other filamentous forms were recorded.

The writer wishes to acknowledge his indebtedness to Dr. H. B. Ward, who suggested a study of the lakes in the Pike's Peak region and the comparison of *B. coloradensis* with *B. Lindahli*, who furnished the material of *B. Lindahli* collected by Professor Nelson, and who has given invaluable assistance in the preparation of these notes.

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Explanation of the Plates.

The figures are original drawings from material preserved in formalin. The outlines and some details were made with the assistance of the Abbé camera lucida, and the drawings were completed at the time from the same specimens.

Plate X.

Fig. 1. Posterior end of the body of a larva of *C. coloradensis* showing the developing caudal appendages. × 119. At this stage the body mass was unsegmented and the first and second antennæ and mandibular legs alone could be distinguished.

Figs. 2–6. Developing caudal appendages of *B. coloradensis*.

Fig. 2. At the time when six body segments can be seen. × 119.

Fig. 3. At the time when twelve body segments can be seen. × 119.

Fig. 4. At the time when all abdominal segments can be distinguished. × 119.

Fig. 5. Later stage. × 35.

Fig. 6. A still later stage. × 35.

Fig. 7. Male second antenna or clasper of *B. Lindahli; ta*, toothed area. × 20.

Fig. 8. Male second antenna or clasper of *B. coloradensis; t*, tubercle; *ta*, toothed area. × 20.

Fig. 9. Distal end of the male second antenna of *B. Lindahli* viewed from the front. × 35.

Fig. 10. The same viewed from the outside. × 35.

Fig. 11. The same viewed from the front and below. × 35.

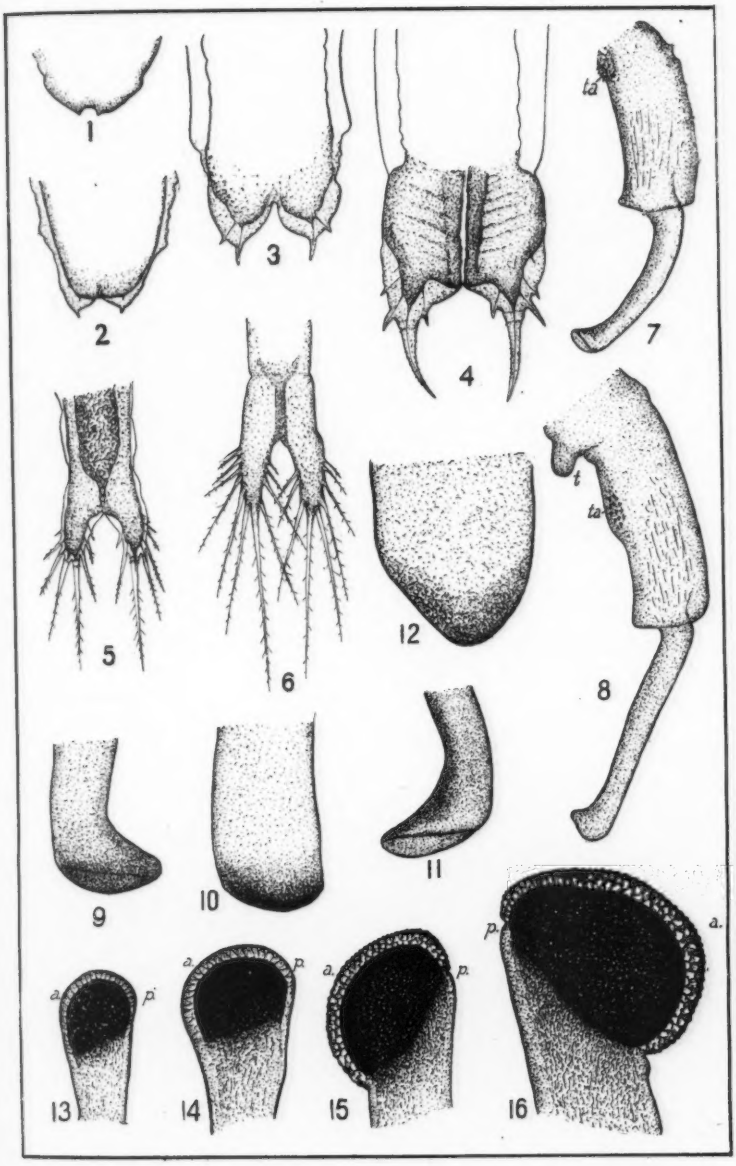
Fig. 12. Distal end of the male second antenna of *B. coloradensis* viewed from the outside. × 35.

Fig. 13. Eye of the female of *B. Lindahli*, dorsal view; *a*, anterior side; *p*, posterior side. × 35.

Fig. 14. Eye of the male of *B. Lindahli*, dorsal view; *a*, anterior side; *p*, posterior side. × 35.

Fig. 15. Eye of the female of *B. coloradensis*, dorsal view; *a*, anterior side; *p*, posterior side. × 35.

Fig. 16. Eye of the male of *B. coloradensis*, dorsal view; *a*, anterior side; *p*, posterior side. × 35.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ta
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PLATE XI.

FIG. 17. Ovisac of *B. Lindahli* with eggs. × 24.
FIG. 18. Ovisac of *B. coloradensis* with eggs. × 24.
FIG. 19. Caudal appendages of male of *B. coloradensis*. × 21.
FIG. 20. Caudal appendages of female of *B. coloradensis*. × 21.
FIG. 21. Caudal appendages of male of *B. Lindahli*. × 21.
FIG 22. Caudal appendages of female of *B. Lindahli*. × 21.
FIG. 23. Head of male of *B. coloradensis*, front view. × 14.
FIG. 24. Head of male of *B. Lindahli*, front view. × 14.

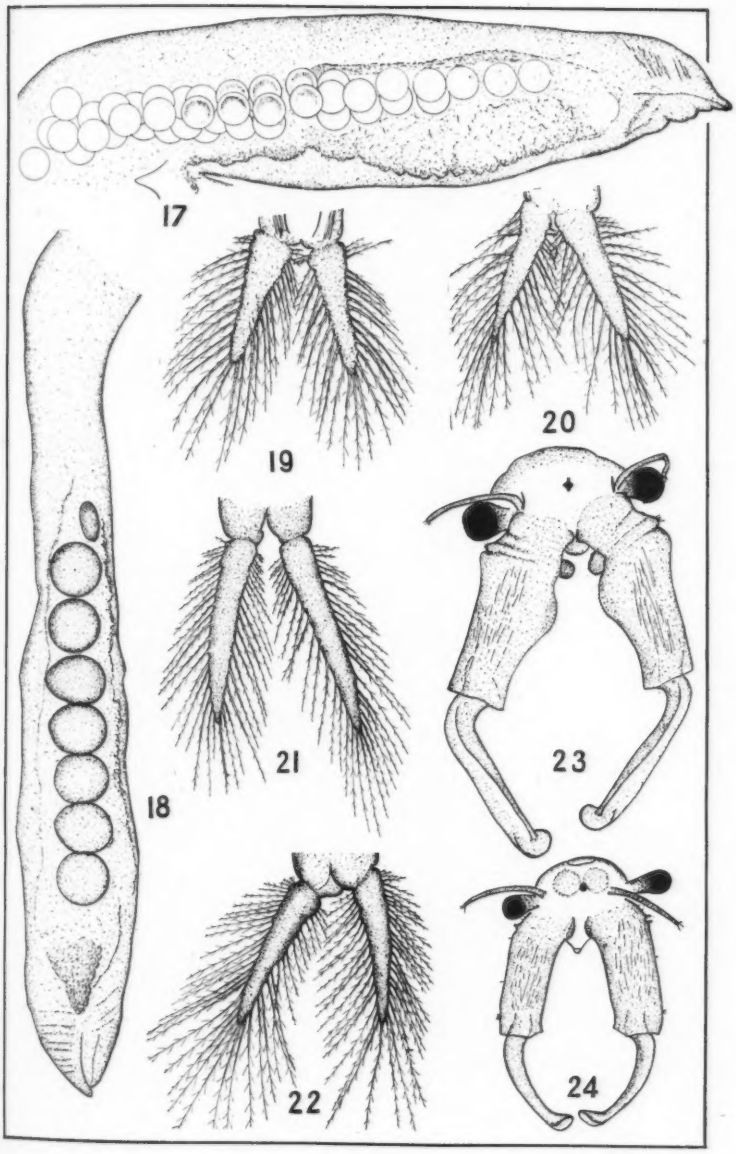
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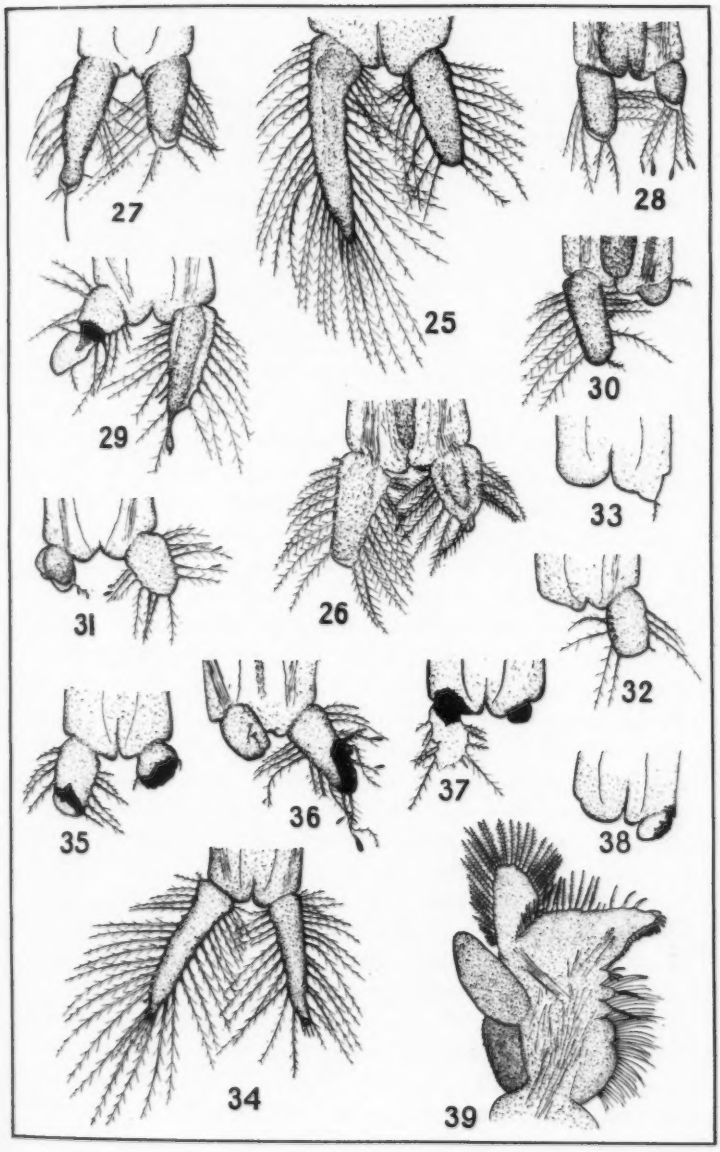
PLATE XII.

FIGS. 25–28, 30–33. Caudal appendages of females of *B. coloradensis* in various stages of absorption. × 25.

FIGS. 29, 34–38. Diseased caudal appendages of females of *B. coloradensis*. × 25.

FIG. 39. Branchial leg of the sixth pair of the male of *B. Lindahli*. × 14.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